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Some studies on a previously undescribed fungus on Juniperus scopulorum Sarg

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SOME STUDIES ON A PREVIOUSLY UNDESCRIBED FUNGUS ON JUNIPERUS SCOPULORUM Sarg.

by

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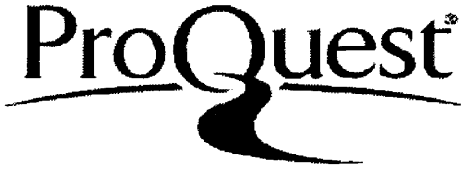

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TABLE OF CONTENTS

Page

I. LIST OF FIGURES v

II. STATEMENT OF PROBLEM 1

III. INTRODUCTION AND HISTORICAL BACKGROUND 2

IV. DISTRIBUTION 4

V. APPEARANCE OF THE DISEASE 6

VI. STRUCTURE AND DEVELOPMENT OF THE PATHOGENE 8

The Thyriothecium, 8
The Presence of Pycnidia, 13
The Mycelium, 13

VII. TAXONOMY OF THE PATHOGENE 15

Comparison with Seynesia Juniperi & Stigmatea sequoiae, 18
Description of Species, 22

VIII. EXPERIMENTAL 23

Culture Methods, 23
Sporulation Inducement Methods, 25
Host Inoculations, 26

IX. EXPERIMENTAL RESULTS 28

Growth in Culture, 28
Sporulation Inducement results, 29
Host Inoculations, 30

X. DISCUSSION 31

Origin of the Ascocarps, 31
Spore Germination, 32
Hyphopodia, 32
Stroma versus Thyriothecium, 33
Nutrition, 35
Comparison with Seynesia Juniperi & Stigmatea sequoiae, 36
Culture Studies, 37
Development of the Disease, 39

Page

XI. SUMMARY 41

XII. BIBLIOGRAPHY 43

XIII. FIGURES 46

LIST OF FIGURES

Figure		Page
1.	Distributional Map of the Disease	47
2.	Heavily-infected Tree From Lolo Creek	48
3.	Heavily-infected Tree From the East Side of Flathead Lake.	49
4.	Germinating Ascospores Showing Hyphopodia	50
5.	Hyaline Ascospores Germinating 'in situ'	51
6.	Mycelial Formation of Ascocarps	52
7.	Superficial Mycelium and Ascocarp Initials	53
8.	Cross-section of Coalesced Thyriothecia	54
9.	Asci Discharged from Ascocarp	55
10.	Ascospores Germinating 'in situ' and Showing Conjugation	56
11.	Top View of Ascocarp Showing Pseudo-ostiole	57
12.	Cross-section of Pycnidium	58
13.	Superficial Mycelium and Ascocarp Initials	59
14.	Cross-section of Ascocarp Showing Hyphal "Feet"	60
15.	Cross-section of Ascocarp of Seynesia Juniperi	61
16.	Habit View of Seynesia Juniperi	62
17.	Top View of Ascocarps of Stigmatea sequoiae	63
18.	Cross-section of Ascocarp of Stigmatea sequoia	64
19.	Cross-section of Ascocarp of Seynesia sp. nov.	65
20.	Lightly-infected branchlet of Juniperus scopulorum	66
21.	Pycnidial Growth In Culture	67

STATEMENT OF PROBLEM

Rocky mountain juniper (Juniperus scopulorum Sarg.), bearing black minute fruiting-bodies upon their branchlets, were first observed by Dr. Charles W. Waters of Montana State University, in the summer of 1946 along the Blackfoot River, 14 miles northeast of Missoula, Montana. Further observations have, since then, disclosed this fungus to be present in a number of other areas in western Montana, and in 1951 it was also found at the Northern Rocky Mountain Experiment Station at Priest River, Idaho. The fungus was studied and tentatively identified as an Ascomycete belonging to the family Microthyriaceae. A comparison of this fungus with known species occurring on other members of the genus Juniperus, and closely related genera, indicates that it has not been previously described. As a result, studies were conducted by the writer in an attempt to classify and to determine the life history of this fungus and its physiological relationships to its host.

INTRODUCTION AND HISTORICAL BACKGROUND

The ascocarps of this fungus are brown to brown-black in color, hemispherical to flattened, and seated superficially on the host. Also, the ascocarps do not appear as true perithecia, but rather, like halved perithecia, consisting of a shield-like cover, or scutellum, and a thin-walled base. These characteristics at once place the fungus among the Hemisphaeriales, syn. Microthyriales. The radiate structure of the scutellum, and the scantiness, or sometimes absence, of the thallus, according to the classification of Stevens & Manter (1925), who followed the classification of Theissen and Sydow, further identifies the fungus as a member of the family Microthyriaceae.

Saccardo supplied the first description of the family and situated it in the order Dothidiales.

Lindau, in Engler's 'Die Pflanzenfamilien' (1897), includes the Microthyriaceae in the order Perisporiales.

According to Orton (1924), Höhnel limited the Microthyriaceae to include only those forms showing the "inverse-radial" arrangement, and termed the fructifications of this group thyriothecia. Following this work, Theissen erected the order Hemisphaeriales which he divided into three families, including those genera having a superficial, halbert to shield-shaped perithecium in the family Microthyriaceae.

Arnaud removed the Microthyriaceae from this order and set it

in a separate order, the Microthyriales.

Atkinson (1915) considered the Microthyriales to be reduced forms, derived on the one hand from Sphaeriales and on the other from Phacidiales and possibly some from the Perisporiales. He also states that, although the Microthyriales have usually been placed among the Perisporiales, they have little in common.

Doidge (1920), Ryan (1923), and Luttrell (1941), follow Theissen's classification in their work, including the family in the order Hemisphaeriales.

DISTRIBUTION

Since its discovery along the Blackfoot River this fungus has been found in a number of areas in western Montana. A map of Montana, west of the Continental Divide, has been included and is marked to show the locations of known infection areas. (Fig. 1)

Two areas, which contained infected junipers, were noted along Lolo Creek. One tree, found close to the creek, was observed to have a heavy coverage of ascocarps upon its branchlets. (Fig. 2) Only one infected juniper was found along Mill Creek, near Frenchtown, Montana, although a more extensive survey of this area would probably disclose further infections. A rather dense stand of junipers located just outside the city limits of Missoula, along South 3rd Ave., was found to contain but a small percentage of disease-free trees. These junipers, located but a short distance from the Clark Fork River, were, as was the case with all other infected junipers noted, on a site in which rather high humidity could be expected to prevail. A survey, made along Highway 10, east of Missoula, failed to disclose any infections on junipers inhabiting dry south exposures. Only one group of infected junipers was found along this route, and it was located on relatively level terrain and in close proximity to the Clark Fork River. Sufficient evidence has not been gathered to state definitely that this fungus can only become established in areas where rather high

humidity prevails, yet the possibility is certainly present. Many junipers planted in the city and suburbs of Missoula, as well as a heavy planting of junipers in the arboretum of Montana State University, have been inspected and found to be free of this disease. Some factor other than moisture may be the controlling one in this case.

Certain areas have only recently shown evidence of infection. Junipers along the east shore of Flathead Lake, which have been under observation since 1946, were first observed to be infected during 1952. At the same time infections were first noted in the Seeley Lake region, in the South 3rd Ave. grove, and in a number of other areas. Some of these areas could very likely have become infected at an earlier date and gone unobserved up until the present time. Coupled with this increase in range there has been an increase, during 1952, in the intensity of the disease in the older areas of infection. The number of infected trees had apparently increased as had the number of fungal fructifications found on the individual tree. It is interesting to note that during the past winter the fungus continued to be active, germinating ascospores being found frequently during this period. Following such a favorable winter it seems probable that a further increase in intensity of this desease can be expected during 1953.

APPEARANCE OF THE DISEASE

The first evidence of infection is noted with the appearance of small, black, dot-like bodies which are discernable to the naked eye. These are the ascocarps, or thyriothecia[1], of the fungus. When only a few of these "dots" appear on a branchlet they may be confused with dust or soot particles, though the writer has found the reverse to occur more often. The majority of the thyriothecia are found upon the upper surface of the branchlets, though they are by no means confined to that area.

There are no external signs, such as necrotic or chlorotic areas, accompanying a light infection. Also, viewed microscopically, the cells of the host show no signs of disorder. It is only after the disease becomes intensified, with the individual branchlets bearing a profusion of ascocarps, that the leaves show a chlorotic condition. (Fig. 2 & 3) The deep green coloration of the leaves fades and eventually turns brown. It is well to note here that the study of the external effects of this disease has been greatly complicated by the fact that heavy infestations of red spider (Tetranychus telarius) found on practically all junipers examined have caused similar fading and browning of the

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[1] The term "thyriothecia", which shall be used throughout this paper to denote the ascocarp of this fungus, is attributed by Orton (1924) to Höhnel who applied it to the fructifications of the Microthyriaceae. The term has since gained wide useage among mycologists working with this group.

leaves.

Only a few trees were found during 1952 which appeared to be lacking in vigor as a result of infection by this fungus. The branchlets of these trees were heavily covered by the ascocarps and ascocarp initials of this fungus. (Fig. 2&3) More extensive browning of the branchlets has already been noted this spring. During May of this year, in the oldest known area of infection, the Blackfoot area, the foliage of a number of diseased junipers was observed to be noticeably browned in spots. Also, during this month, new areas of infection were found along the west shore of Flathead Lake (Fig. 1) which contained a number of junipers whose branchlets were extensively browned. The light brown color of these dying branchlets stands out rather vividly against the naturally green foliage of the juniper.

It appears that junipers of all ages are susceptible to infection from this fungus. Some of the largest junipers encountered, approximately 18' in height, as well as seedlings down to a height of 6", have been found bearing the ascocarps of this fungus. It is then obvious that the juvenile foliage, as well as the mature type of foliage, is subject to the attacks of this fungus.

STRUCTURE AND DEVELOPMENT OF THE PATHOGENE

The Thyriothecium

Thyriothecia were found to originate in two different ways: from hyphopodium-like cells formed by germinating ascospores; and from mycelial cells.

In the first method of origin the ascospore germinates to form a 1-celled hyphopodium which varies in shape from almost circular to three-lobed. (Fig. 4) This cell is dark brown in color and is surrounded by a relatively thick, almost black wall. The hyphopodium then gives rise to one or more cells which may duplicate it in coloration, or assume a much lighter hue. These cells in turn give rise to other cells which are always lighter in color than the hyphopodium. There is no set pattern to this growth. In some cases the hyphopodium gives rise to a group of cells which, through further "budding", eventually surrounds it, the ascospore collapsing and finally disappearing. Or, the hyphopodium will give rise to a new cell which will bud on one or more sides, eventually forming a tightly-knitted group of cells with the hyphopodium positioned on the rim, and in which the ascospore remains intact, or partially so, for some time. In either case, the group of cells finally elongates radially through further addition of out-growing cells or the addition of radiating strands of hyphae. These cells, or hyphal strands, coalesce; the cells becoming divided by short dark cross-septa, to form a radiate shield, or scutellum, which is flattened against the leaf.

Doidge (1942) states, that in the genus Asterina, the spore commonly germinates to form first a hyphopodium from the base of which two or more hyphae grow to form the superficial mycelium. A number of instances have been noted, in the present studies, in which either the hyphopodium has given rise to the hyphae, or hyphal branches have emerged from the spore in close proximity to this cell. In some cases the hyphopodium has not appeared; instead a germ tube has emerged from the ascospore to form a short, branched mycelium. (Fig. 5)

In the second method of origin of the ascocarp a cell of the mycelium gives rise to one or more closely-appressed cells; these may be darker in color than the rest of the hyphae. Three cells, their bases tapered and closely appressed, sometimes originate from the mycelial cell. (Fig. 6d) These cells, in turn, give rise to new ones which radiate out from the point of origin. When only one lobe is formed from the mycelial cell, it is usually followed by radiating hyphal branches (Fig. 6c) which may bud in a dichotomous manner. These hyphal branches usually originate from mycelial cells which are adjacent to that cell from which the lobe originated.(Fig. 7) In either case, these cells coalesce laterally, are divided tangentially by dark septa, to form the disk-like scutellum. Ward (1382), in his description of the formation of the perithecia of Asterina spissa Syd., notes that the radial walls in the disk result from the coalescence of the out-growing lobes side by side; the tangential walls being true septa.

Ascocarp initials are commonly found originating along the borders of more mature ascocarps. Also, groups of initials may originate in

close proximity to one another from other initials formed by ascospore hyphopodia which give rise to short hyphal branches, and, in turn, upon which other initials form. In cross-section this characteristic produces the effect of the ascocarps having two, or more, locules. (Fig. 8) In plan view a group of such coalesced ascocarps may be differentiated by their individual "ostioles". As many as five ascocarps in one group have been observed to be so fused together.

In either method of development the thyriothecium is formed inversely, that is, from the underside of the hyphopodium or mycelium (Fig. 6a,b) although this is not always apparent. According to Ryan (1926) Raciborski was the first to state that the perithecia of the Microthyriaceae were inverse, with Theissen, Doidge, Arnaud, and von Höhnel accepting this as correct. Ryan, in her own studies, has also verified this manner of origin.

The scutellum, thus initiated, consists of brown, radially-arranged rows of pseudoparenchymatous cells which are flattened against the leaf. Soon afterward, the shield becomes carbonous, losing its radiate appearance, except at the margins. The radiate character of the margin may, in turn, become more or less obscured; in which case, it can usually be observed by simply crushing the ascocarp. Ryan (1926) states that by boiling the specimen in a KOH solution the radiate structure can be seen more clearly. At maturity the ascocarp is circular with a crennate margin, and ranges in size from 160-300u. In cross-section it is hemispherical to half-oval in shape with an extremely thin-walled, dark base which eventually disintegrates. The structure of the base is not

apparent. The walls of the scutellum are usually 2-3 cells thick at maturity.

Prior to attaining full growth, the scutellum is no longer found to be appressed against the leaf surface. The central portion of the disc has undergone an upheaval, leaving only its periphery in close contact with the leaf. While undergoing this upheaval, slender hyaline hyphae appear beneath the scutellum. These hyphae increase in number to form a plectenchymous mass. The scutellum continues to expand away from the leaf until it attains a hemispherical to half-oval form. Ward (1882) believes that the increasing mass of hyphae in the cavity of the scutellum forces the disk up in this dome-like manner. Meanwhile, small, spherical cells, which are hyaline in color, have appeared in this mass of hyphae. These are the immature asci, which, as they elongate, form cavities within the plectenchymatous mass. The asci do not all mature at the same time, different stages in development being found simultaneously in the same ascocarp. The mature asci are arranged in a straight basal layer rather than in a fascicle, and are separated from each other by strands of interwoven hyphae. These strands later disintegrate, apparently crushed by the expanding asci, so that the mature asci stand side by side. No paraphyses are present. At maturity the asci are from 60-75u in length (Fig. 9), although some asci have been found which measured 87u. Due to their length they cannot stand upright in the thyriothecium, and are, as a result, forced into a bent position. The asci are oblong to pyriform; when forced into a bent position, reniform with a tapering base. They are usually short-stalked

with the stalk bent at almost a right angle to the ascus proper. Within each ascus eight ascospores are formed which are obovate in outline, and are one-septate, with an indenture at the point of septation. The upper cell is somewhat wider than the lower, being approximately 10u in width, while the lower is generally 8.5u in width. At maturity the spores range from 20-25u in length. They remain hyaline for a rather long period, usually becoming brown when mature. Some spores have been observed germinating while still hyaline in color. (Fig. 5) A rather large oil globule is commonly found within each cell of the spore. Germination may occur within the ascus (Fig. 5 & 10), or, upon discharge from the ascus, may germinate while still within the thyriothecium (Fig. 8), or upon the leaf surface. The spores are released through a pore-like pseudo-ostiole which is formed in the center of the scutellum. Preceding the formation of this opening, the area in which this pore will appear becomes lighter in color, losing its carbonized appearance. This area becomes progressively lighter in color until the opening occurs. Ryan (1926) states that the appearance of this "ostiole" is due to the gelatinization or cracking of the central cells at the point of insertion on the mycelium or hyphopodium. Luttrell (1940) attributes the formation of the longitudinal opening in the ascocarp of Morenoella quercina to the pressure exerted upon this area by the growing asci. This does not seem to be the case here, rather, the "ostiole" seems to form by a disintegration, or gelatinization, of the central cells. The pore-like character of the opening appears to bear this out. (Fig. 11 & 19)

The Presence of Pycnidia

Sections of diseased juniper branchlets made during various times of the year revealed pycnidia to be present along with the ascocarps of the fungus. These pycnidia are superficial to semi-erumpent, sub-globose to almost oval in shape, and range in size from 45-70u in diameter. (Fig. 12) The base of the pycnidium appears to be of an evanescent nature. Hyaline to brown, when mature, 1-celled pycnidiospores are produced in quantity within the pycnidium. They are approximately 5 x 3.3u in size; their mode of attachment to the pycnidial walls has not been determined. In top view the pycnidium is seen to be circular and composed of an indefinite mass of brown hyphae. Immature pycnidia are yellowish-brown in color, rectangular-like in shape, and composed of a network of small, almost square, cells.

There is no definite proof at hand which would connect this asexual stage with the perfect stage of the fungus under study; however, many of these pycnidia have been found adjoining thyriothecia, seemingly connected to them. Also, the mycelium which is associated with the pycnidia is very similar to that associated with the thyriothecia.

The Mycelium

The mycelium is in greatest evidence during the fall and winter months. During the summer it is either lacking, or present in very small amounts. In any case, it is never profuse. When present, the mycelium consists of rather short hyphal branches scattered over the leaf surface, although here and there anastomosing hyphae form small networks of mycelium. (Fig. 7 & 13) The hyphae vary in color from

almost hyaline to brown, are rather closely septate, 5-6.7u in width, and 6-8.5u septate. Hyaline to lightly-tinted hyphal branches are commonly found originating from the edges of the thyriothecium, forming a one-celled layer around its border, or branching outwardly from the border for a short distance. Hyphal branches may round off to form short chains of cells, which appear to be able to act as conidia. Two-celled fragments, somewhat similar in appearance to the ascospores, may also be pinched off from the hyphae. These conidial fragments have been found germinating.

The ascocarp is connected to the host by means of hyphal "feet" which penetrate the cuticle of the host. (Fig. 14) Spherical to elongated, hyaline hyphae appear beneath the cuticle at an early stage in the development of the ascocarp. Individual hyphae may progress downward, for a short distance, between the epidermal cells. Penetration of the epidermal cells by these hyphae has never been seen in the large number of sections examined. In a few cross-sections there would seem to be a connection between the hyaline sub-cuticular hyphae and the hyphal "feet". However, in the majority of cases, no actual connections between the two have been noted.

TAXONOMY OF THE PATHOGENE

The main division made by Stevens & Ryan (1939) in their key to the genera of the Microthyriaceae, is in regard to the presence or absence of free mycelium. Some difficulty arises in the interpretation of this division, for there is probably some superficial mycelium present, at one time or another, in most of the fungi classified as having 'no free mycelium'. Ryan, in reply to an inquiry by the writer as to what constituted free mycelium stated that "free mycelium refers to superficial mycelium", and added, "When the ascocarps appear on the surface there is little or no surface mycelium." Luttrell (1941) in describing Myiocopron smilacis, a fungus characterized by an absence of free mycelium, mentions the mycelium of branching hyphae formed upon germination of its ascospores. Those genera characterized by the presence of free mycelium, to use Asterina, Morenoella, and Lembosina as examples, may be said to be typified by a rather dense, more or less reticulately arranged mycelium. The distinction may then be said to be one made between those members of the Microthyriaceae having a scanty superficial mycelium of a temporary nature, and those having a rather dense superficial mycelium. Doidge (1920) in her description of the general characteristics of the Microthyriaceae of South Africa, refers to the mycelium of this family as being either persistent or evanescent.

The mycelium of the fungus under study was found to be of an evanescent nature such as that described by Doidge, appearing in varying

amounts at different times, though never becoming profuse. Thus, according to the preceding interpretation, this pathogene may be classified under the division in which free mycelium is said to be absent. The fact that the spores are 2-celled and dark, and the ascomata smooth rather than setose, places this fungus in the genus Seynesia Sacc.

In connection with these studies the writer corresponded with a group of well-known workers in the field of pathology. Specimens of the fungus were forwarded to each of these pathologists with the request that they give their opinions as to its generic placement. Specimens containing only hyaline spores, and probably all immature, were examined by Ryan[1] who stated that if the spores are brown at maturity then the fungus should be placed in the genus Seynesia. Miller[2], Davidson[3], and Wehmeyer[4], expressed similar opinions as to its generic placement and added that it might be the species, Seynesia Juniperi (Desm.) Stev. n. comb.

The genus Seynesia Sacc. is described by Engler & Prantl (1897) as being characterized by a fruiting body that is superficial, smooth, shield-shaped, growing together at the border with the sub-stratum, with central opening; asci oval to oblong, 8-spored. Spores oblong, 2-celled, septate, brown. Paraphyses none. The genus has been described elsewhere as an Asterina without persistant, or free, mycelium and as a Microthyrium with brown spores.

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[1]Ryan, Mary Hilaire: Dept. of Bot., Rosary College, Ill.
[2]Miller, Julian H. : Dept. of Path. & Plant Breed., Univ. of Ga.
[3]Davidson, Ross W. : Bureau of Plant Industry, Div. of For. Path., Colorado A & M, Fort Collins.
[4]Wehmeyer, L. E. : Dept. of Bot., Univ. of Mich.

The type species is Seynesia nobilis (Welw. et. Curr.) Sacc. and is found on palms in Africa. Petrak (1927) would not include the genus Seynesia in the Microthyriaceae as he believes the type species is identical with Steganopycnis oncospermatis and is a typical Sphaeriaceae. For those Microthyriaceae with radiate shield, a central pore, and 2-celled brown spores, Petrak established the genus Arnaudiella. The genus Ferrarisia Sacc. as amended by Petrak includes the forms with 2-celled spores previously known as Seynesia spp.

Clements & Shear (1931) lists the genus Seynesia with the synonyms Arnaudiella Petr., Ferrarisa Sacc., and Seynesiola Speg., under the family Microthyriaceae and, also, under the family Sphaeriaceae with the synonym, Steganopycnis. Doidge (1942) accepts Petrak's classification and applies the generic name Ferrarisa to a new species. However, in their monograph of the Microthyriaceae, Stevens & Ryan (1939) continue to use the genus Seynesia. Stevenson (1943) applied the generic name Seynesia to a newly described species of the Microthyriaceae. The genus Seynesia is still, then, recognized as a member of this family by some mycologists.

The fungus described herein shall be placed tentatively in the more established genus, i.e., Seynesia.

Comparison with Seynesia Juniperi and Stigmatea sequoiae

Two fungi have been described which appeared similar in enough respects to the fungus under study to warrant mention by some of the mycologists contacted. Seynesia Juniperi (Desm.) Stev. n. comb., syn. Microthyrium Juniperi Sacc. and Stigmatea sequoiae (Cke. & Hark.) were the species so mentioned.

Seynesia Juniperi is described in Rabenhorst's Kryptogamen-Flora (1887) under the generic name Stigmatea with the following list of synonyms: Dothidea Juniperi Desmaz., Gibbera Juniperi Auersw., Stigmatea alpina Spegazz., and Microthyrium Juniperi Sacc. No mention was made of the genus Seynesia. The perithecium of the species was described as hemispherical or broadly conical, often with papille, upon a rather solid or coarse, cuticular substance, brown-black, naked, about 200u in diameter. Asci oblong or short cylindric to sac-form, short stipitate, 8-spored, 60-70u long, 16-18u thick. Spores oblong, 2-celled, tied in the middle, under cell somewhat smaller, brown when mature, 20-25u long, 7-9u wide. Found upon the needles of Juniperus communis. As described in North American Pyrenomycetes (1892) the sizes were: perithecia, 200-300u; asci, 60-70 x 20u; spores, 16-25 x 6-8u. The hosts were listed here as Juniperus virginiana and Sequoia gigantea.

The generic placement of this fungus seems to be in doubt, if judgment may be based upon the different genera in which it has at one time or another been included. In Krytogamen-Flora (1887) it is stated that in no case should this species be accorded to Microthyrium. Yet, it certainly appears to be more closely related to the family

Microthyriaceae than to the family Stigmataceae. An examination of Microthyrium Juniperi on Juniperus californica, from the Bonar collection, shows the ascomata to be seated superficially upon the leaf, and not sub-cuticularly as is characteristic of the family Stigmataceae. The ascoma is connected by means of hyphal "feet" to a seemingly discontinuous layer of dark-brown sub-cuticular hyphae. (Fig. 15) This sub-cuticular hyphae appears to be that which is referred to in Krytogamen-Flora (1887) as the substance upon which the ascomata are seated. This mycelial layer is by no means a stroma, and cannot be likened to the sub-cuticular stroma upon which the ascocarps of Stigmatea robertiani are seated. The material examined by the author did not contain mature spores. If they are brown in color upon maturity, the fungus would seem to be a Seynesia. Stevens & Ryan (1939) placed this species in the genus Seynesia.

A comparison of Seynesia Juniperi with the organism under study reveals that structually the two fungi are similar in many respects. The shape and size of the asci and the ascospores are very much alike. In cross-section the shape of the perithecia are similar, and the size, as given by Ellis & Everhart (1892), is similar. However, there are a number of ways in which the perithecia differ. First, the papille, which is described as often found on Seynesia Juniperi, is not characteristic of the organism under study. A description of Stigmatea Juniperi (Desm.) in N. A. Pyren. (1892) does not, however, have any mention of the perithecia of this species being papillate. The material examined by the author did not appear to be papillate, although this

may be due to the time at which the material was collected, or to its age and dried condition. This then, may or may not, be a point of difference. Secondly, the sub-cuticular layer of hyphae, already described, differs greatly in color and abundance from the sub-cuticular hyphae found in the organism under study. The importance of this difference lies in the fact that the characteristics of the mycelium, both external and internal, have been used in the Hemisphaeriales to differentiate between families, and within the Microthyriaceae to differentiate between genera. Also, in her description of Seynesia cordiae sp. nov., Ryan (1924) lists as one of the characteristics of the species the presence of an internal mycelium which is hyaline in color. Thirdly, there seems to be a difference in the manner in which the ascocarps of the two fungi are arranged upon their respective hosts. An examination of Juniperus californica revealed the ascocarps of S. Juniperi to be clustered on individual leaves (Fig. 16), rather than scattered over entire branchlets in the manner of the ascocarps of the fungus under study. (Fig. 2 & 3) Based on these differences, and probable difference, with emphasis on the difference in sub-cuticular hyphae, the two fungi are thought to be different species of Seynesia.

Stigmatea sequoiae (Cke. & Hark.), syn. Dothidea sequoiae (Cke. & Hark.) is described in N. A. Pyren. (1892) as found on leaves of Cupressus and Librocedrus decurrens. The size and shape of the asci and ascospores are again quite similar to those of the fungus under study, except that the spores are described as hyaline. Very little additional information is given concerning the species. The type collection of this species was

obtained through the generosity of Mr. J. T. Howell of the California Academy of Sciences; the host being Librocedrus decurrens. This material contained only hyaline ascospores, as described. Examination of the ascocarp revealed a fimbriated border to be sometimes present. (Fig. 17) Further description is unneccessary, for the two fungi on these points alone are definitely dissimilar. It should be noted here that this species should be further studied to determine its correct taxonomic position in the Hemisphaeriales. According to Stevens (1925), the characteristic which places the members of this order in the family Stigmataceae is that the ascomata be sub-cuticular. In the material examined neither the ascomata nor the hypothallus were found to be immersed. (Fig. 18) The superficial ascomata and scanty mycelium would seem to place this fungus in the family Microthyriaceae.

A survey of the literature dealing with members of the Microthyriaceae, and related forms, revealed two species which appeared to be quite similar to the fungus under study. Comparisons made between these fungi revealed the existance of the aforementioned dissimilarities. As a result, it is concluded that the fungus concerned is a new species, and, it is henceforth so described.

Description of Species

Seynesia _____ sp. nov.

Ascocarps circular with crennate margin, superficial, radiate, becoming carbonous, 160-300u in diameter. Hemispherical to half-oval in cross-section. Ascocarps scattered singly or coalescing in scattered groups. Free mycelium evanescent, never profuse. Internal mycelium hyaline. Asci pyriform to clavate, short-stalked; 60-75u in length; 8-spored; aparaphysate. Spores long hyaline, becoming dark, 2-celled, the top cell larger than the lower, indented at the septa; 20-26u in length, top cell 10u in width, lower cell 8.5u in width. Pseudo-ostiole pore-like.

On Juniperus scopulorum. Western Montana and the Northern Rocky Mountain Experiment Station at Priest River, Ida.

EXPERIMENTAL

Culture Methods

Growth in culture of members of the Microthyriaceae has been recorded in only a few instances. Horne (1905) reported that all attempts to culture Lembosia Rolfsii failed, and indicated that this species was probably a strict parasite. Growth on artificial culture-media has been noted by Fisher (1939) as having been recorded for only one species of this family. The species cultivated was not named. At a later date, Luttrell (1940) found that Morenoella quercina was easily induced to grow on artificial media. Of the various types of media used, he found malt agar to be the most successful. The same author (1941) was also successful in obtaining Myiocopron Smilacis in culture on both malt and potato dextrose agar media.

The method most commonly used in the attempts to isolate this organism in culture was the removal of ascocarps from the host with an aseptic needle followed by their placement upon the medium being used. The use of surface disenfectants, i.e., HgCl 1:1000, and alcohol, even for short periods, resulted in negative growth. Single spore isolations were attempted, notably by Ezekiel's Mosification of Keitts method (1930) using the following modification: Mature ascocarps were mashed in a drop of water which had been placed upon a cover slip. The cover slip was then inverted over a Van Tiegham cell and the extruding ascospores were allowed to germinate. Upon germination, a streak culture was

prepared in a Petri dish of agar. Following Ezekiel's method, single spores were then located on the agar plate and cut out with an included disk of agar. At this point, in each attempt, the spore was lost to sight in the disk of agar and no growth was initiated.

Various media were used in attempting to culture this organism, namely, Bonar's Modification of Leonian's Agar Medium, Czapek's Culture Solution with agar, Honey Agar (Fisher, 1939), Zike's Sucrose Asparagin Solution with agar, Juniper Dextrose Agar, and Juniper Agar. Juniper Agar medium and Juniper Dextrose Agar medium are basically juniper branchlet decoctions; the ingredients and procedure used in their concoction being as follows:

Juniper Agar Medium

Juniper branchlets	200.0g
Agar	30.0g
Distilled water	1500.0cc

Fresh juniper branchlets were put through a coarse grinder, then steamed in 750cc of distilled water for 1½ hours. Agar was then dissolved in the remaining water which had been heated. Both solutions were then mixed, filtered, and autoclaved at 15 pounds pressure for 15 minutes.

Juniper Dextrose Agar Medium

Juniper branchlets	100.0g
Dextrose	30.0g
Agar	30.0g
Distilled water	1500.0cc

Steam ground juniper branchlets for 1 hour in 750cc of distilled water; filter and add dextrose. Dissolve agar in remaining 750cc of water. Mix solutions, filter, and autoclave for 15 minutes at 15 pounds pressure.

Methods to Induce Sporulation

In an attempt to induce sporulation, some of the cultures were subjected to varying conditions of temperature, to aging, and, during the latter part of this study, a few cultures were subjected to ultra-violet radiation. Also, transfers were made from some of the cultures to Petri dishes containing water-agar plus chopped juniper branchlets in a further effort to induce fruiting.

Cultures obtained on Honey Agar and Juniper Dextrose Agar were transfered to test tubes containing the same medium. Half of the test tubes were then placed at room temperature while the other half were placed in a cold room in which the temperature remained at approximately 40° F.

Two Petri dishes, containing cultures maintained on Honey Agar were both half covered by heavy paper. Prior to their placement over the dishes, the papers were wiped with a cloth dampened with 1:1000 HgCl to prevent contamination. The uncovered portion of the cultures were then subjected to 10 and 15 minutes of ultra-violet radiation respectively. The covered halves of the cultures acted as the checks in this case. Also, two cultures maintained on Juniper Agar were each exposed to a 20 minute dose of these rays. As before, appropriate checks were maintained. The source of light used in both experiments was a 0.75 amp. germicidal lamp. In all cases, the cultures were placed at a distance of 11.5 cm. from the light source. Prior to exposure the Petri dish covers were removed.

Three Petri dishes containing water-agar plus chopped juniper

branchlets were inoculated from cultures growing on Honey Agar medium. The procedure used in the preparation of this 'natural' medium was the same as described by Hansen & Snyder (1947). Two of the subsequent cultures were left at room temperature while the third, after growth had first been initiated, was placed in a cold room at a temperature of approximately 40° F.

Host Inoculations

In the greenhouse:

Four year old potted Juniperus scopulorum seedlings were inoculated, at various times, using cultures growing on all of the aforementioned media. The inoculum was mashed in a small amount of distilled water and applied over the entire tree, in some cases by means of a camels-hair brush, and in others, by means of an atomizer.

The first group of inoculated trees were placed in pans of water and covered with bell jars. Check plants were sprayed, others were brushed with distilled water; placed in pans of water and covered with bell jars. Later, an "Iceless Refrigerator" was constructed, following the plans of Hunt (1919). Subsequent inoculated seedlings were placed, together with their checks, within this inoculation chamber. Inoculated trees were left in their respective inoculation chambers for a period of three weeks, undergoing frequent examination during this time.

Three healthy junipers were placed in the inoculation chamber with a potted diseased juniper. Examination of the ascocarps found on this juniper revealed the presence of mature ascospores. The humidity was maintained at a high level and the trees were left in this condition

for a period of two weeks. Also, a number of junipers were placed under bell jars with diseased branchlets bearing mature spores. The diseased branchlets were placed in contact with the branchlets of the healthy junipers. Finally, one healthy juniper was placed, and left to remain for over four months, in the same room in which the diseased trees were kept.

In the field:

Mycelia from the various cultures was separately macerated in distilled water. The inoculum from each culture was then spread over separate branches of three, apparently disease-free, junipers growing in the field. Each inoculated branch was then covered with cotton; the cotton then dampened with distilled water and covered with a plastic bag. Checks were sprayed with distilled water and covered in a similar manner. At the end on one week half of the inoculated branches were cut off, examined, and placed under bell jars with their cut ends immersed in water.

EXPERIMENTAL RESULTS

Growth in Culture

Bonar's Modification of Leonian's Agar Medium

Initial growth was white and aerial, followed immediately by a surface and surmerged growth of dark mycelium; growth 1½ inches in diameter at the end of two weeks. Growth in flask covering the substratum and appearing as a rather thick stroma. Microscopically the hyphae are brown, cylindrical; forming dark-walled chlamydospores. One-celled hyaline to brown spores were noted which appeared to form from the mycelium. No fruiting structures were noted.

Czapek's Culture Solution with agar & Zike's Sucrose Asparagin Sol. with agar

No growth was initiated when these media were inoculated with the ascocarps of the fungus.

Honey Agar

Rapid growth; covering the Petri dish within two weeks. Aerial mycelium light brown to brown. Surface and surmerged mycelium dark brown. Hyphae is dark walled; cells almost round in shape and surrounded by a gelatinous-appearing sheath. Medium becoming covered by a dark stroma-like growth. No fruiting structures appearing.

Juniper Dextrose Agar

Growth moderate. Mycelium dark brown, cells cylindrical; forming radiating rings of alternately sterile, then fruiting, areas upon the surface of the medium; radiating outwardly from the point of inoculation.

Fruiting structures cylindrical-upright, up to 4mm in height, tomentose, sclerotium-like in hardness, sterile. One-celled spores, hyaline to brown, found massed on the hyphal branches.

Juniper Agar

Slow growth, but this is offset by the relative absence of contamination. Mycelium light brown, dark-walled, cylindrical, finally covering the medium with a dark stroma-like layer. Minute black pycnidia appearing on the surface of the mycelium. Pycnidia 45-60u in diameter, pseudoparenchymatous in structure; brown. Spores hyaline, oval, 4-5.5 x 3-3.5u. A light brown aerial mycelium found initiating on many of these cultures has been observed producing pycnidia similar in structure to those produced on the surface mycelium, but attaining a diameter of 300u; pycnidiospores oval, hyaline to light brown, 5-7 x 3.5u.

Sporulation Inducement Results

No sporulation resulted in the cultures growing on Honey Agar in either the cold room or at room temperature. Cultures maintained on Juniper Dextrose Agar produced the structures, already described, when placed under either temperature condition.

The cultures exposed to ultra-violet radiation did not, as a result, produce any fruiting structures. The aerial mycelium, in all cases, was either killed or stunted, depending upon the amount of radiation to which it was exposed.

No fruiting structures were produced upon the water-agar-juniper branchlet media which had been inoculated from a Honey Agar culture and

left at room temperature. Also, as could be expected, growth was slow on this medium. The culture which had been placed in the cold room developed at an even slower rate than those left at higher temperatures. However, approximately four weeks after inoculation, minute black bodies appeared on the mycelium of this culture. (Fig. 21) The exact time of their appearance is not known, for the plastic bag in which the Petri dish was enclosed obscured, to a certain degree, the details of growth. The fruiting bodies were found, in most cases, to be sterile, in others, to contain oval hyaline spores. Fruiting structures 150-250u in diameter, black; their structure indistinguishable until crushed, then pseudoparenchymatous in nature. The mycelium was very similar in appearance and size to that found on the surface of infected junipers.

Host Inoculations

No positive results were obtained in any of the inoculations made. Even the junipers placed in contact with diseased trees failed to produce any of the symptoms of the disease.

DISCUSSION

Origin of the Ascocarps

The origin of the ascocarps of those genera of the Microthyriaceae having free mycelium present (not evanescent) has been extensively studied by Ryan (1926), who listed four characteristic ways in which these structures were initiated. These methods were: from a cell of the mycelium; from hyphopodia (mycelial); from a short lateral branch; from a nodulate cell. The first method was noted as being the most common. Only two species having nodulate cells on the mycelium were studied by Ryan, and in both of these the perithecia did not actually develop from the nodulate cell itself, but from a cell of the mycelium. Only two methods of origin of the ascocarps were noted for the fungus under study; the method of development from spore-hyphopodia being the only one not previously described. The scantiness of the mycelium made this study difficult, and, for this reason, it is entirely possible that other methods of origin were overlooked. Doidge (1942) mentions the formation of hyphopodia by germinating ascospores, but found that a short mycelial branch was also initiated. From this mycelial branch the ascocarps were, in some manner, initiated.

Spore Germination

The ascospores were found to germinate in two ways: by the production of a germ tube; by the formation of a hyphopodium. In studies of sections cut parallel to the leaf surface it was observed that the method of germination by the formation of a hyphopodium exceeded the other method by far. On the other hand, observations made of spore germination in hanging drops of water revealed the reverse to be true; more spores germinated to form germ tubes than to form hyphopodia. Also, spores germinating while still within the ascus usually formed germ tubes. It appears possible that the method of germination may hinge on moisture conditions, the formation of germ tubes requiring more moisture than is needed in the formation of the hyphopodium.

Spores placed in hanging drops of water germinated within a period of five hours. The germ tubes were hyaline, and originated, in the majority of cases, from the lower cell of the ascospore. It was not uncommon, however, to find germ tubes emerging from the top cell only, or from both cells of the same spore. Both hyaline and brown spores were noted to germinate when placed in water. One germ tube was observed to reach a length of 56u, at which time it was 3-septate, still hyaline, and forming short branches.

Hyphopodia

Doidge (1942), in describing the hyphopodium which was sometimes formed during spore germination, did not attribute any function to it other than that its base was the point from which arose two or more hyphae. The mycelial hyphopodium, however, has been the subject of a

great deal of conjecture as to its probable function. Ryan (1926) cites a number of writers who variously suggested that the mycelial hyphopodia in Meliola functioned as sexual organs, organs of absorption, and organs of absorption and attachment, and the cytological studies of Arnaud which seemed to indicate that the hyphopodia in the Microthyriaceae acted as organs of absorption and attachment.

Structures have been seen, in sectional mounts of diseased junipers, which, due to their dark coloration and size, are thought to be hyphopodia formed from germinating ascospores. These structures initiated short hyphal branches, or "feet", which penetrated the cuticle of the host. If these structures are truly hyphopodia, as they appear to be, then it is likely that they act also as organs of attachment for the initiating ascocarps.

Stroma versus Thyriothecium

The shield-shaped, pseudo-ostiolate, and radiate (except for the family Hemisphaeriaceae) fructifications of the Hemisphaeriales have been regarded by some mycologists as the lower half of inverted perithecia. The upper portion, or shield, is then regarded as the morphological base while the lower portion, or that part of the ascocarp from which the asci arise, is regarded as the top of the perithecium. The term thyriothecium was applied by Höhnel to this type of fructification, and has since gained wide usage among mycologists working with this group of fungi. Doidge (1920, 1942) regards the ascocarps of these fungi as thyriothecia.

Many mycologists, on the other hand, regard these fructifications as stromata within which the asci are formed. Ward (1882) in his investigations of Asterina spissa Syd. terms the disk a stroma which

develops prior to the formation of the true sexual organs. Orton (1924) cites Arnaud as naming the base from which the asci arise in Protothyrium Salvadorae (Cooke) Arn., the ascus-stroma. Orton adds that this is no true stroma, but that we need further evidence as to the possible existance of ascogonia and their distribution before judging its real relationships. The ascocarp of Morenoella quercina is described by Luttrell (1940) as a flat stroma consisting of two parts; a pseudoparenchymatous cover, or scutellum, and a plectenchymatous inner portion.

In the present studies there is no direct evidence to indicate that the scutellum is other than vegetative in origin. In fact, most evidence points toward its vegetative origin. However, in two cases, the germ tubes of ascospores germinating in hanging drops of water were observed to conjugate (Fig. 10) in a manner typical of some of the smuts. Also, the mycelium, when present, displays anastomosing to a high degree. Whether this fusion of hyphae is sexual in character has yet to be determined. Here then, we have two ways in which sexual union may occur. Their significance is, however, doubtful, for studies on the origin of the ascocarps failed to disclose any such sexual union occurring prior to their initiation. It must be concluded, then, at the present time, that the ascocarp is probably vegetative in character, and is, as a consequence, stromatic in origin. The term thyriothecium is incorrectly applied to this fructification if it is truly stromatic in origin. It has been used by the writer, however, because of the familarity of the term to many readers, and because of its continued

use by some mycologists.

A number of authors of rather recently published references on mycology tend to accept the view that the fructifications of this group are stromata rather than some form of perithecium, e.g., Bessey, "Taxonomy and Morphology of the Fungi", 1950; Alexopoulos, "Introductory Mycology", 1952; Westcott, "Plant Disease Handbook", 1950. On the other hand, Wolf & Wolf, "The Fungi I", 1947, appear to accept the other point of view.

Nutrition

The absence of haustoria or intracellular hyphae in the host tissues raises the question of the manner in which this fungus obtains its nutrients. A number of species included in the Microthyriaceae have been observed to form haustoria. In his studies of Asterina spissa, Ward (1882) observed the occurrance of haustoria which penetrated the epidermal cells of the host. All parts of the haustoria were hyaline while the hyphae from which they originated were brown. Luttrell (1940) could find no evidence of penetration of the host cells by the hyphae of Morenoella quercina, and supposed that nutrients diffused through the leaf cells to the mycelium. The superficial mycelium of Lembosia Rofsii was not seen by Horne (1905) to penetrate below the cuticle. The mycelium of the fungus under study was seen, at times, to extend downward for a short distance between the epidermal cells. The subcuticular hyphae, with the exception of the hyphal "feet", were hyaline. The possibility exists that hyaline absorptive organs, such as described

by Ward, may be present, but be very difficult to discern. However, as no such organs were noted in the sections examined, it must be assumed that a condition similar to that found in Morenoella quercina exists here, and that the nutrients are probably obtained by the fungus through the diffusion of solutes through the epidermal cells.

Comparison with Seynesia Juniperi & Stigmatea sequoiae

The fungus under study has been determined to be a member of the genus Seynesia Sacc. (Farrarisia Petrak) in the family Microthyriaceae. A survey of the literature dealing with the members of this family, and related forms, plus an actual comparison between this fungus and two similar fungi, convinces the writer that the fungus under study is a previously undescribed one. A comparison made between this fungus and Seynesia Juniperi (Desm.) Stev. n. comb. establishes the existance of one major and two minor differences. The major point of dissimilarity between the two lay in their internal mycelium; one being hyaline, and spherical to slightly elongated; the other being brown, elongated and much more profuse. That this can be considered enough of a dissimilarity in itself to warrant the erection of a new species is borne out by the use of the mycelial characters in the keys to the Microthyriaceae and its genera. The comparison between the fungus concerned, and Stigmatea sequoiae (Cke. & Hark.) brought out a number of dissimilarities existing between the two fungi. It seems necessary to cite only the differences in spore color, and the existence, at times, of a fimbriate border in the one species and its entire absence in the other, to establish

with certainty that these were separate species. The fungus has, therefore, been described as a 'species novum', but left unnamed and so open to discussion.

While under comparison, it was noted that the species Stigmatea sequoiae appeared to be more closely related taxonomically to the family Microthyriaceae than to the family Stigmataceae. The writer, therefore, believes that further study should be made of this species in order to ascertain its correct taxonomic position.

Culture Studies

It is believed that the pathogene has been isolated and grown in culture, although, definite proof is lacking. Single spore isolations have failed to initiate growth upon artificial media. Also, all attempts to infect the host have proven futile, and attempts to infect healthy junipers by association and actual contact with diseased junipers have also failed. This may indicate that the degree of pathogenicity of this organism is slight, although observations made of the recent spread of this disease indicate otherwise. No fructifications have appeared in culture; however, there have been no reports of the sexual stage of members of this family having ever been formed in culture. Luttrell (1941) reported the formation of spermagonia, which were abnormal in structure, in his culture of Myiocopron smilacis. The same author (1940) was able to obtain only a very limited vegetative growth in the culture of Morenoella quercina. He reported that this fungus formed black hemispherical colonies about one centimeter in diameter upon malt agar.

Pycnidia have been found in culture, the spores of which resemble

closely those found on the diseased juniper. Fruiting bodies, most of which appeared to be sterile, were produced on a water-agar medium into which had been placed chopped pieces of juniper branchlets. The preparation of natural media, such as this, is described by Hansen & Snyder (1947). They point out the advantages of the use of natural media for the express purpose of inducing sporulation.

Ultra-violet radiation was used during the latter part of this study in an effort to induce sporulation. Stevens (1928, 1930, 1931) successfully employed ultra-violet radiation to produce the sexual stage in a number of different fungi. While no success was met with its use in the present work, it should be noted that very little time was available to test its use and, it was not, as a result, given a through trial.

Of the various types of media used, Honey Agar (Fisher, 1939), which consisted of 5% honey, 2% agar, and distilled water, produced the most rapid mycelial growth. However, Juniper Agar is to be favored for its absence of contaminating growth, and for the production of pycnidia.

A large number of cultures made over a period of two years, on various types of media, have resulted in similar mycelial growths. The mycelium produced is brown in color, finally covering the medium with a stroma-like growth, and appearing black in color. The growth described by Luttrell (1940) for Morenoella quercina, when grown on malt agar, resembles somewhat the growth obtained here. The obtaining of such similar growths of hyphae over a period of time, with the

use of various media, and under different conditions, is the basis for the belief that this organism has been artificially cultured.

Development of the Disease

Ascospore germination has been noted frequently during the past winter, which was, for this region, a relatively mild one. It indicates, however, that the fungus will usually become active very early in the spring. New infections, then, probably initiate at this time, following a more rigorous winter. The comparative 'abundance' of mycelium found during the winter, as contrasted with that found during the summer months, indicates that this fungus is also vegetatively more active during periods of rather low temperature. Fisher (1939), working with the 'sooty moulds' of Australia, within which she includes members of the Microthyriaceae, found that these fungi have a comparatively low-temperature optimum, i.e., from 15-20°C.

Following infection, the disease progresses slowly upon the host, but for a few exceptions. The 'Flathead Lake Tree' (Fig. 3) within a period of less than one year, had become very heavily covered by the ascocarps of this fungus. The leaves of this tree are already exhibiting a chlorotic condition, ranging from a slight loss of green color, to an actual browning. Sections of diseased juniper branchlets have disclosed no indication of extreme functional disturbance to the leaves as a result of light infections. Only heavy infections of the individual branchlets may induce the conditions as described. Prior to this year only a few trees were seen to be very heavily infected. However, pronounced browning of the branchlets of a number of trees in the

Blackfoot area, and along the west shore of Flathead Lake, has been observed this spring.

SUMMARY

A new fungus on Juniperus scopulorum Sarg., first observed in 1946, has been increasing in range and intensity throughout western Montana. The fungus has been identified as a previously undescribed species of Seynesia Sacc., a genus included in the family Microthyriaceae, order Hemisphaeriales. This species was separated from the closely-similar species Seynesia Juniperi (Desm.) Stev. n. comb., mainly by the differences in their internal hyphae.

The ascocarp is believed, by some mycologists, to consist of a stroma, differentiated into a pseudoparenchymatous cover, or scutellum, and a plectenchymatous inner portion. Others believe the ascocarp to be an inverted perithecium, or thyriothecium. The significance of the two cases in which conjugating ascospores (Fig. 10) have been found, as well as that of the anastomosing character of the mycelium is unknown, but may have a bearing on the question of the origin of the ascocarp.

In the study of the ascocarp's development, a new method of origin has been described. This method of origin is from hyphopodia formed from germinating ascospores. Another method of origin, characteristic of this fungus, has already been described by Ryan (1926).

Pycnidia were observed on the diseased junipers which may be the asexual stage of this fungus. Further studies will have to be made, though, to connect definitely the two fungi.

Attempts to culture this organism have been made under varying conditions of light, moisture, etc. Similar types of growth have been obtained with the use of different media and over an extended period of time. Single spore isolations have proved futile, as have attempts to induce sexual sporulation and to inoculate successfully the host with the culture material.

The effects of this disease on the host are not pronounced unless the tree is very heavily infected, that is, covered with the fruiting bodies of the fungus. When so infected, the leaves of the juniper are noted to become chlorotic, and, eventually, necrotic. This spring there has been a marked increase in the number of diseased junipers exhibiting necrotic symtoms, and lack of vigor. It is believed that this disease may prove fatal to some of the trees whose branchlets are extensively browned. The majority of the infected junipers do not appear to be so severely affected, however, and the disease, to date, does not seem to be a dangerous threat to the juniper population.

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FIGURES

Photography

The following pictures were taken and processed by David Saltsman, Senior, Forestry School, Montana State University.

A 35mm Leica camera and Plus X film was used in all of the photography. All micro-photographs, as well as those taken through binoculars, were taken with the aid of a Leitz Micro Ibso attachment. The photograph used in Fig. 21 was the only one in which no type of magnifier was used.

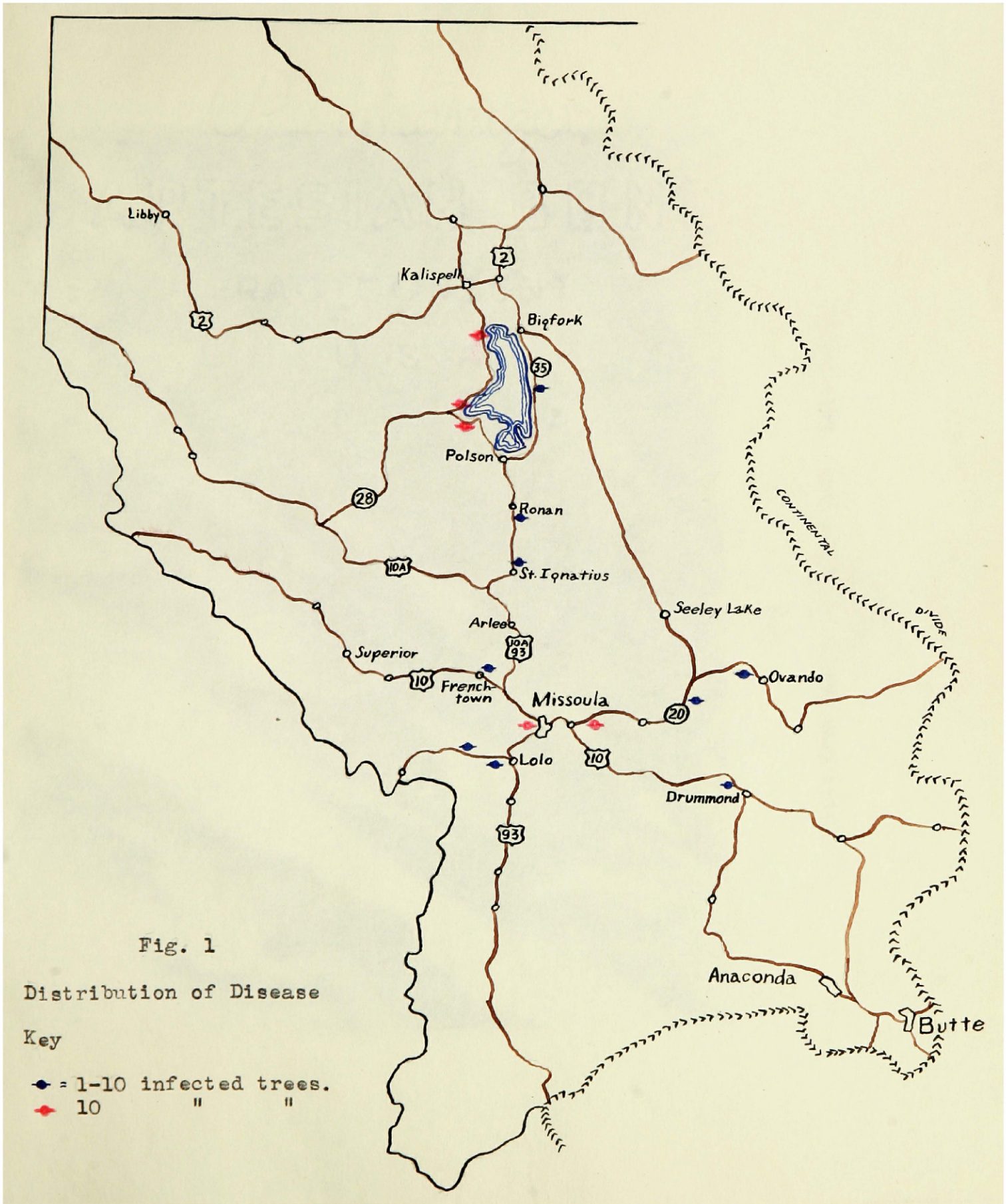

Fig. 1

Distribution of Disease

Key

● = 1-10 infected trees.
● 10 " "

Fig. 2

Branchlets from heavily-infected tree. Lolo Creek, Montana.
(x 30)

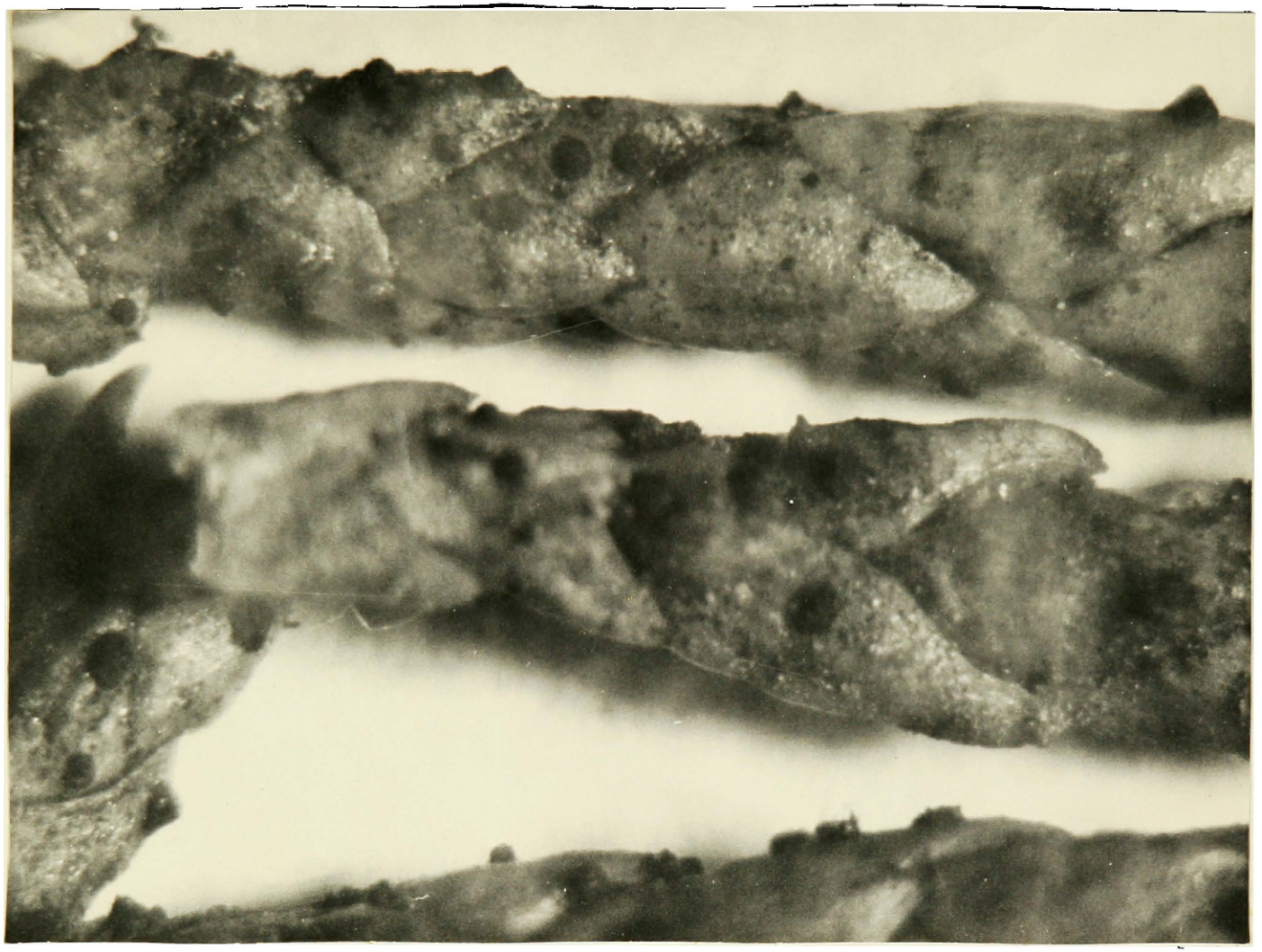

Fig. 3

Branchlets from heavily-infected tree. Flat-head Lake, Montana.

(X 30)

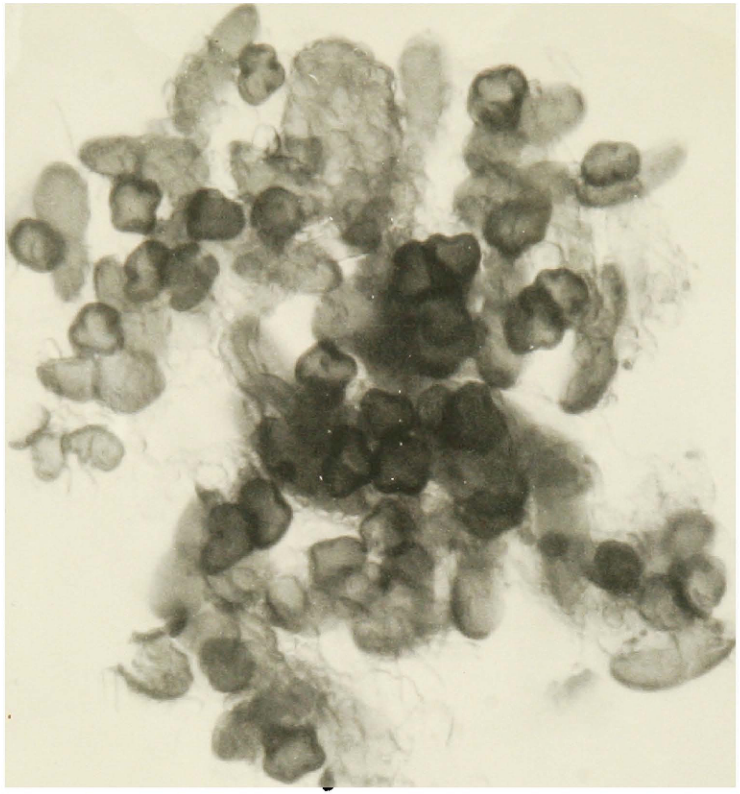

Fig. 4

Germinating ascospores producing hyphopodia.
(x 730)

Fig. 5

Ascospores germinating within the ascus. Note the hyaline spores in top ascus.
(x 820)

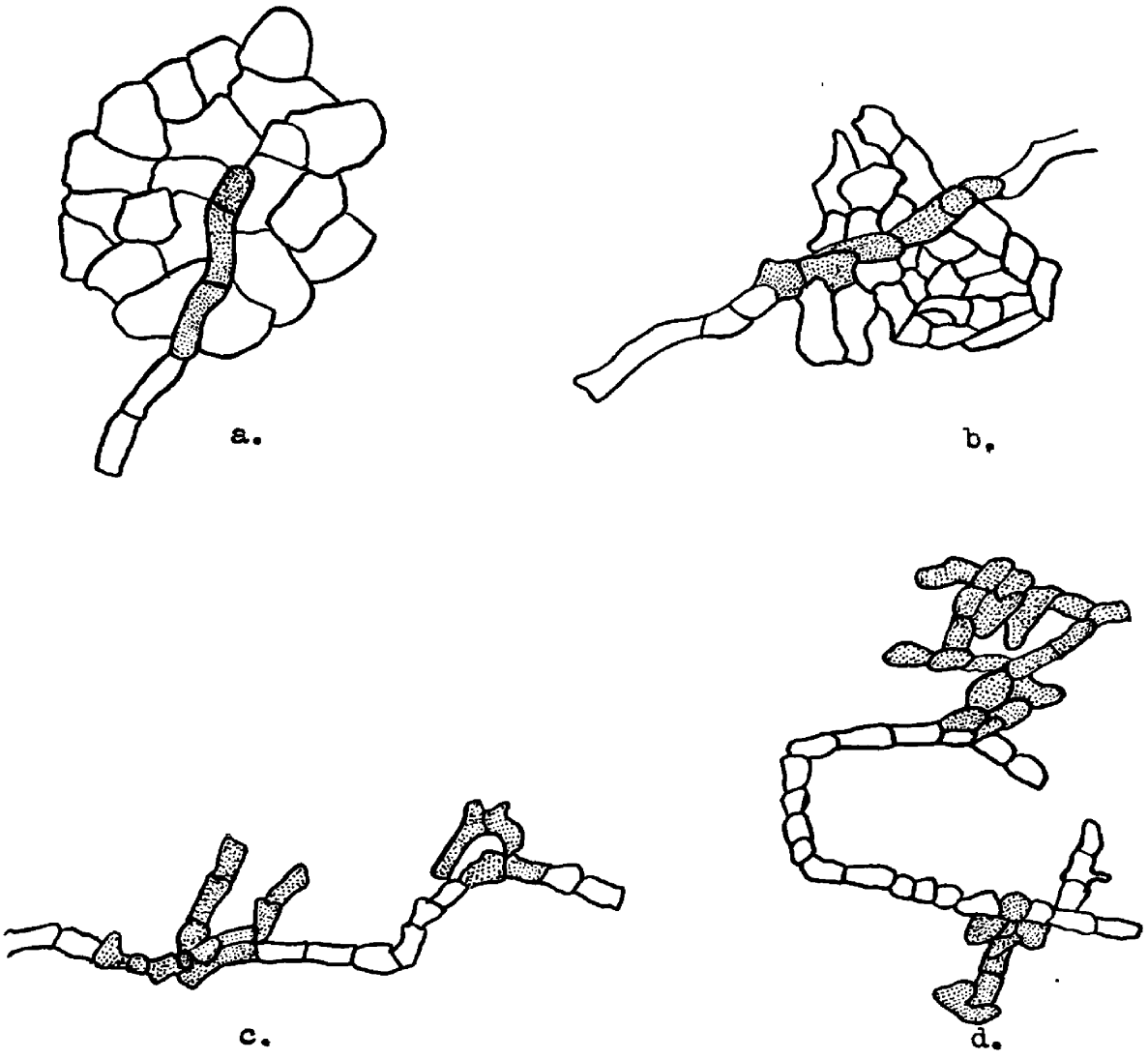

Fig. 6

Mycelial Formation of Ascocarps

a. & b. Note that the ascocarp initials are beneath the hyphae. The hyphae are stippled.

c. & d. Formation of ascocarp initials from "budding", and issuing short hyphal branches, of the mycelial cells. Those cells, appearing to take part in the formation, are stippled.

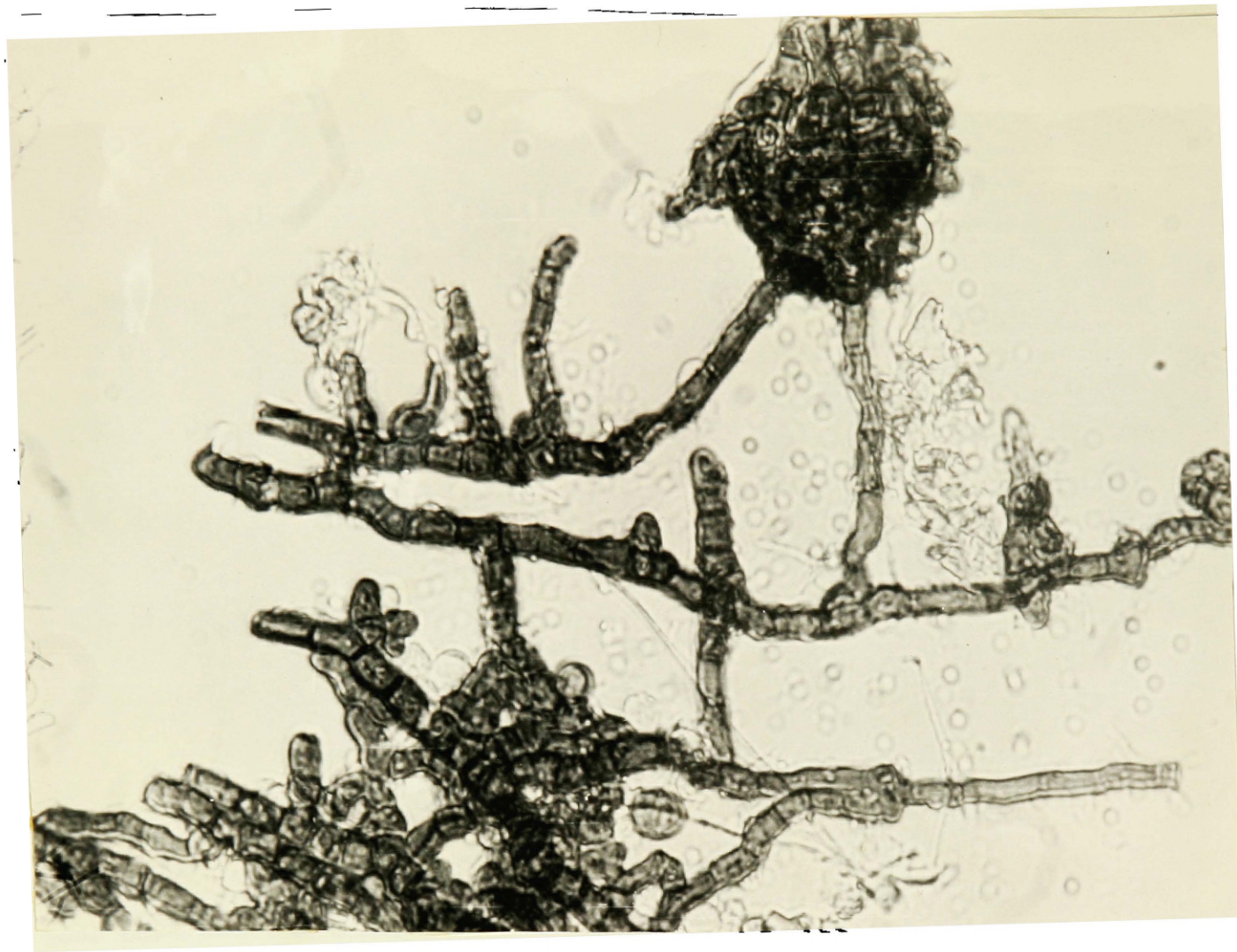

Fig. 7

Superficial mycelium and ascocarps initials.
Note the "budding" on the mycelium and the
short hyphal branches.

(x 670)

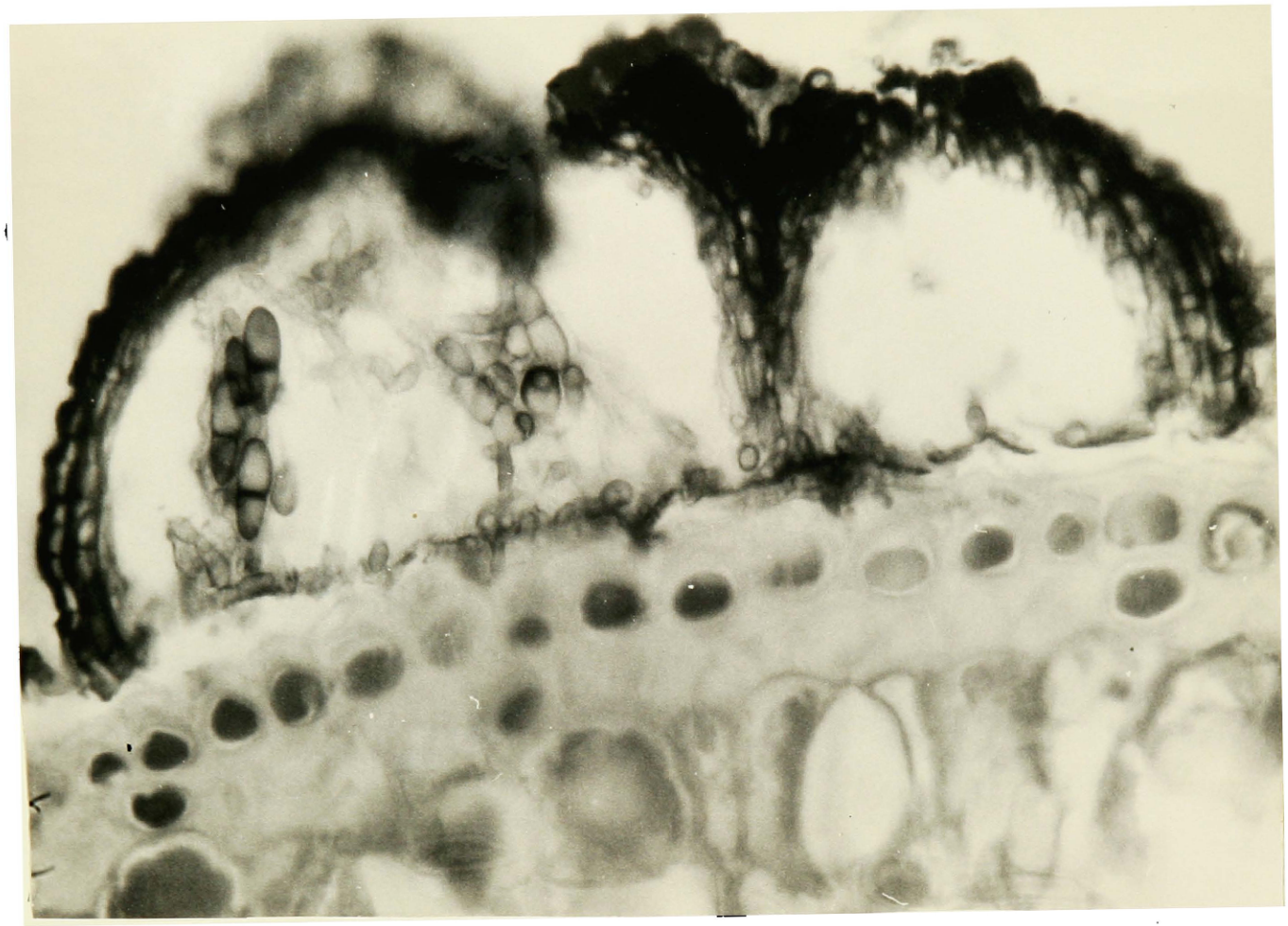

Fig. 8

Section of coalesced thyriothecia. Note the germinating ascospores.
(x 550)

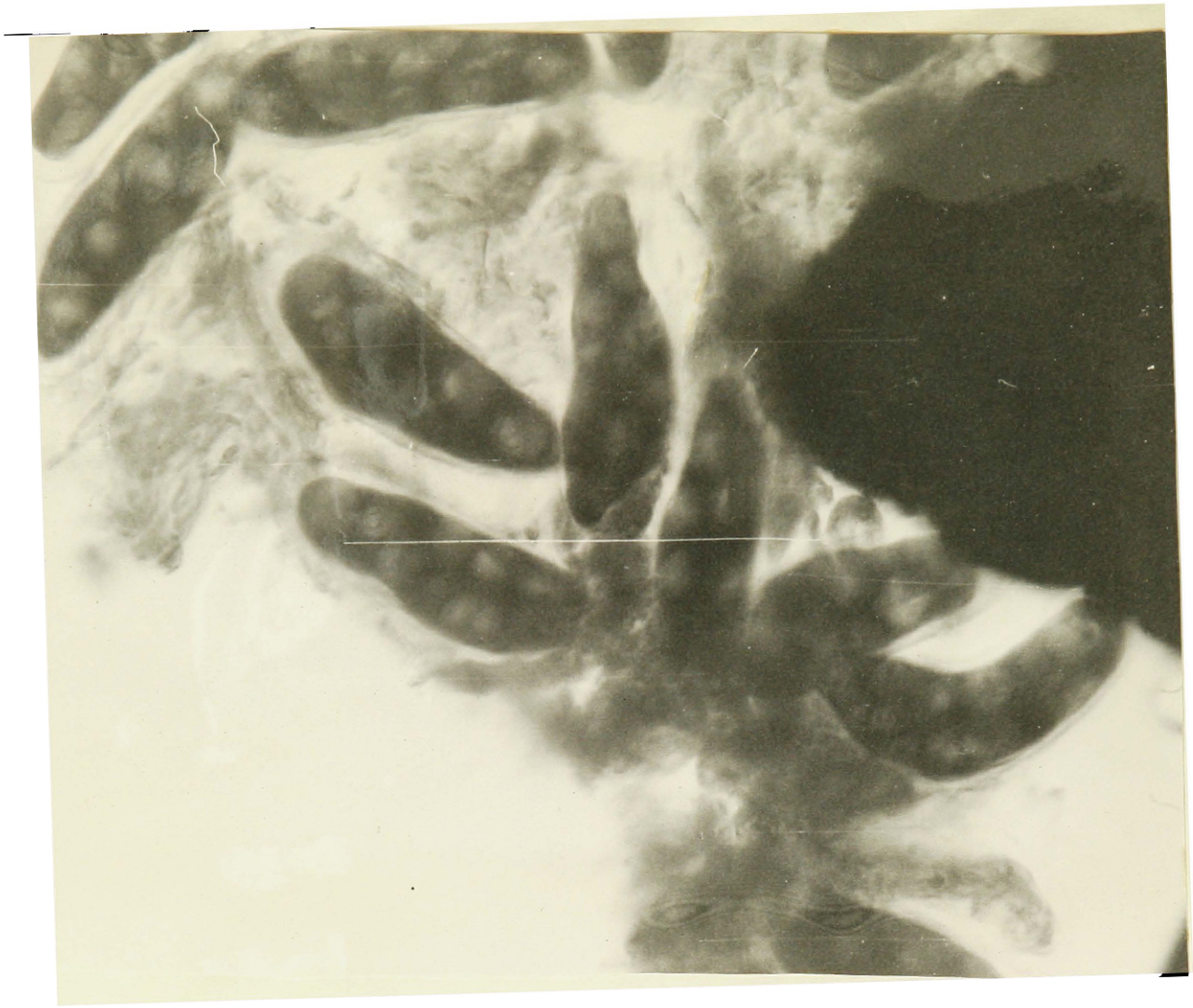

Fig. 9.

Asci, stained with iodine, issuing forth from a crushed ascocarp.
(x 820)

Fig. 10

Ascospores germinating within the ascus. Note the conjugating germ tubes.
(x 900)

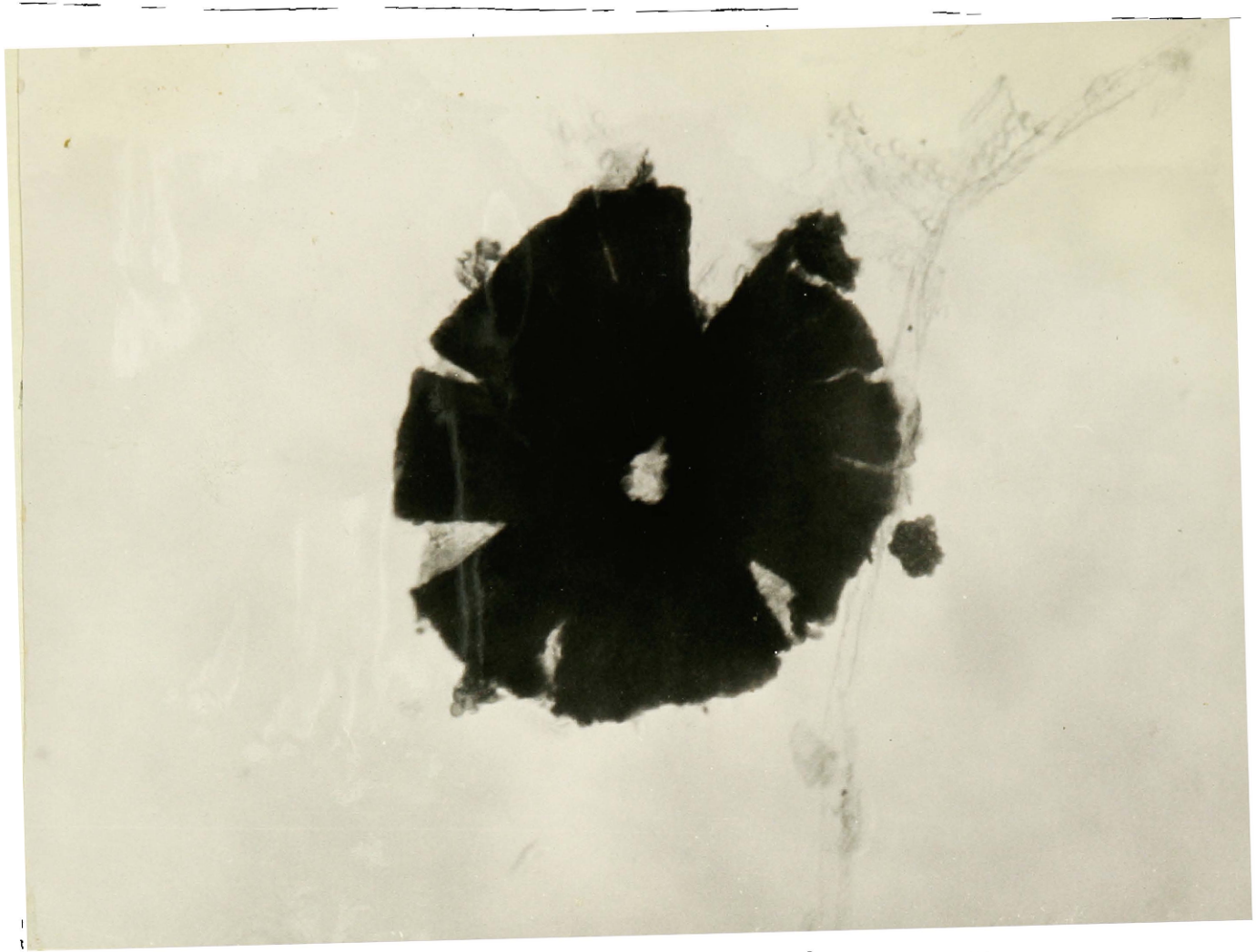

Fig. 11

Top view of ascocarp showing pseudo-ostiole.

(x 530)

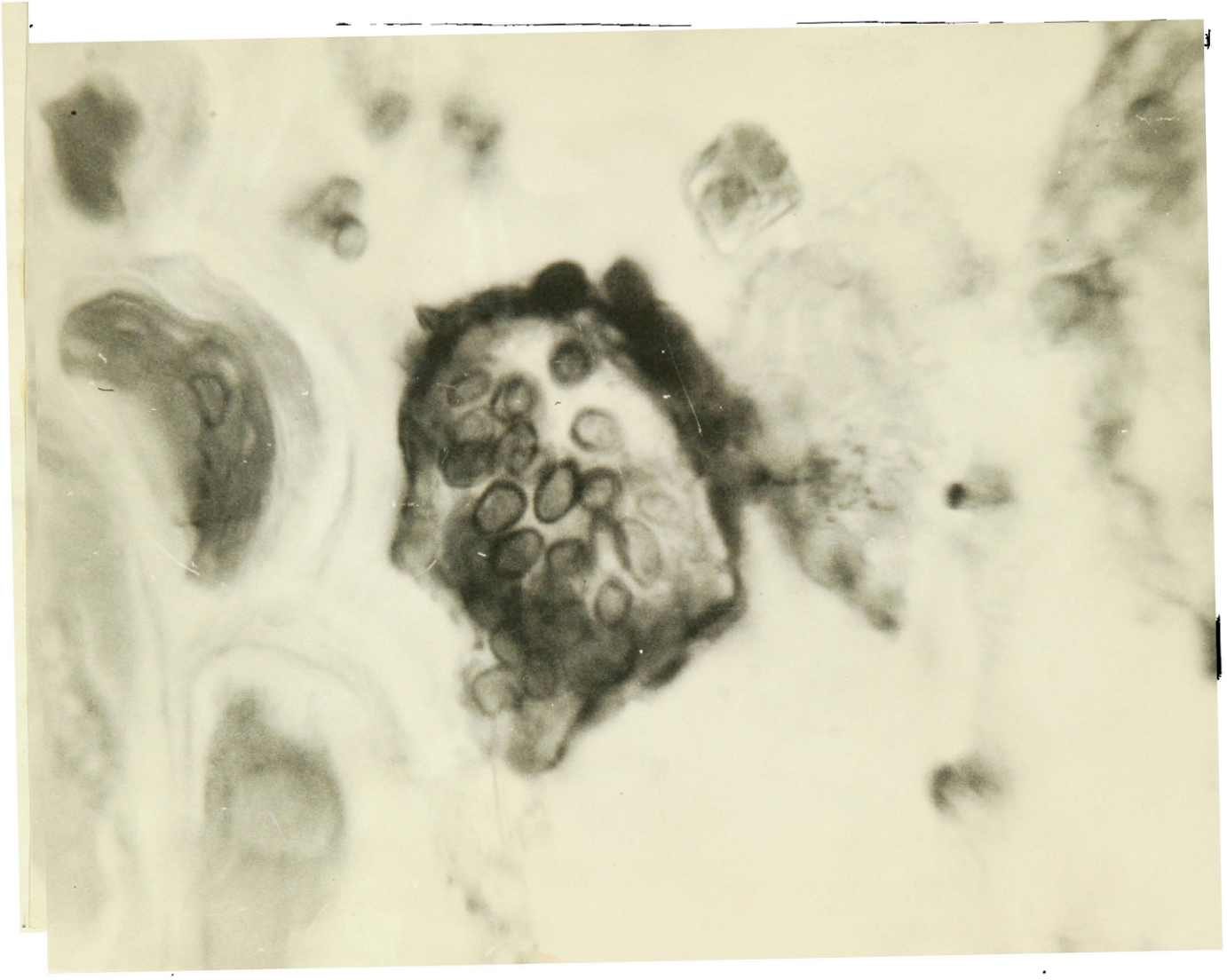

Fig. 12

Cross-section of pycnidium. On leaf of Juniperus scopulorum.
(x 1000)

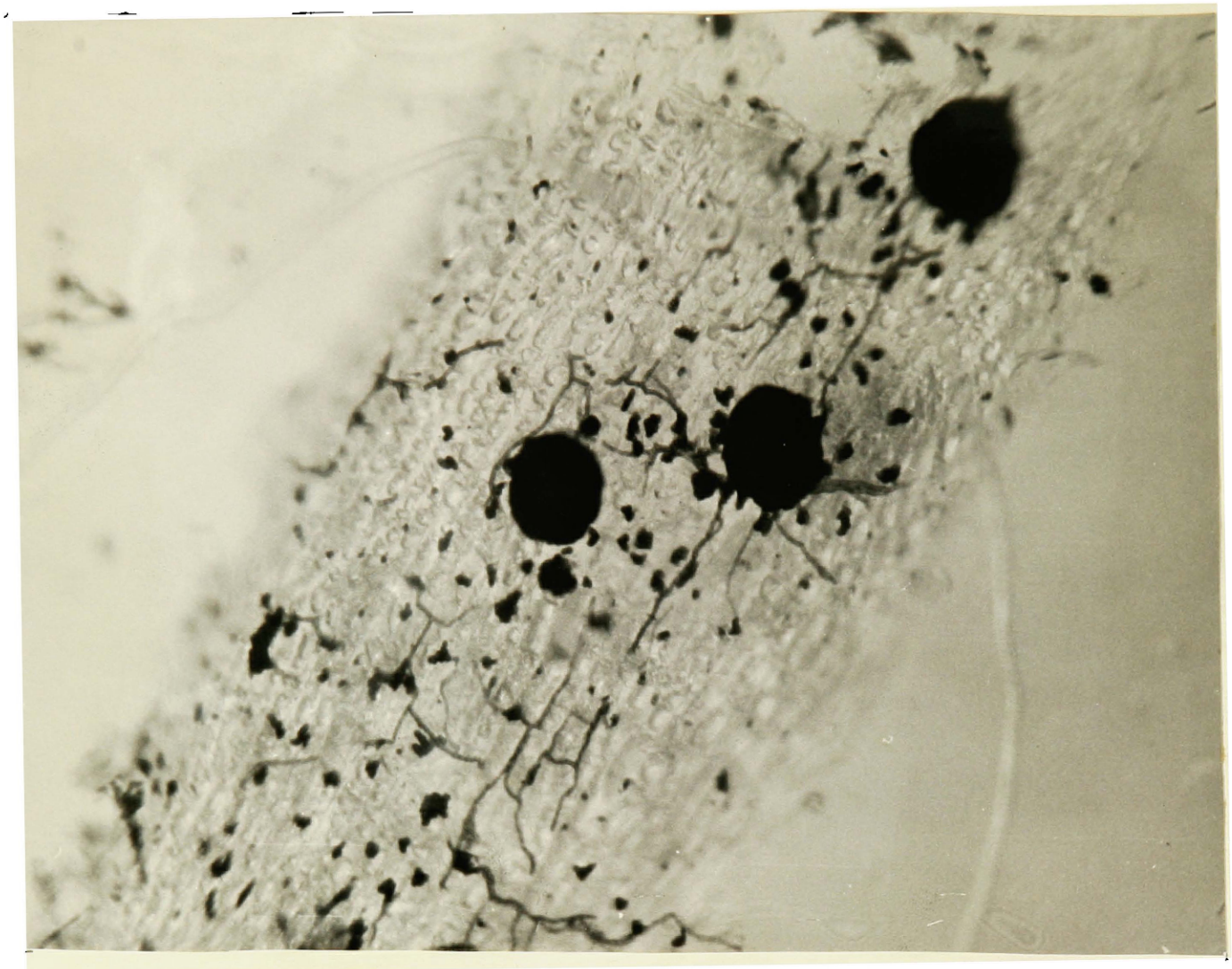

Fig. 13

Section cut parallel to the leaf surface. Note the free mycelium and the ascocarp initials.
(x 80)

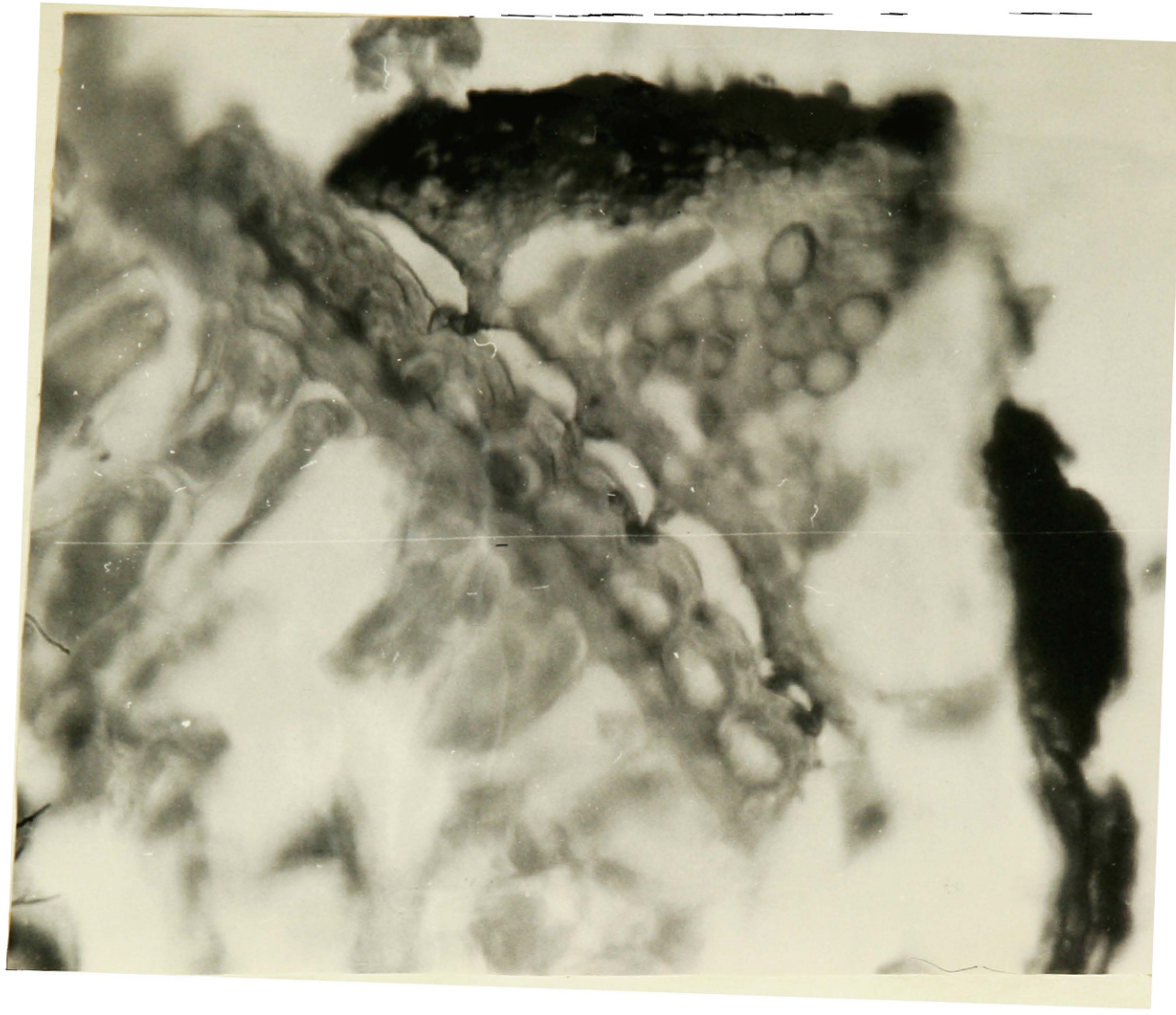

Fig. 14

Cross-section ascocarp, showing the hyphal "feet" penetrating the cuticle of the leaf.

(x 540)

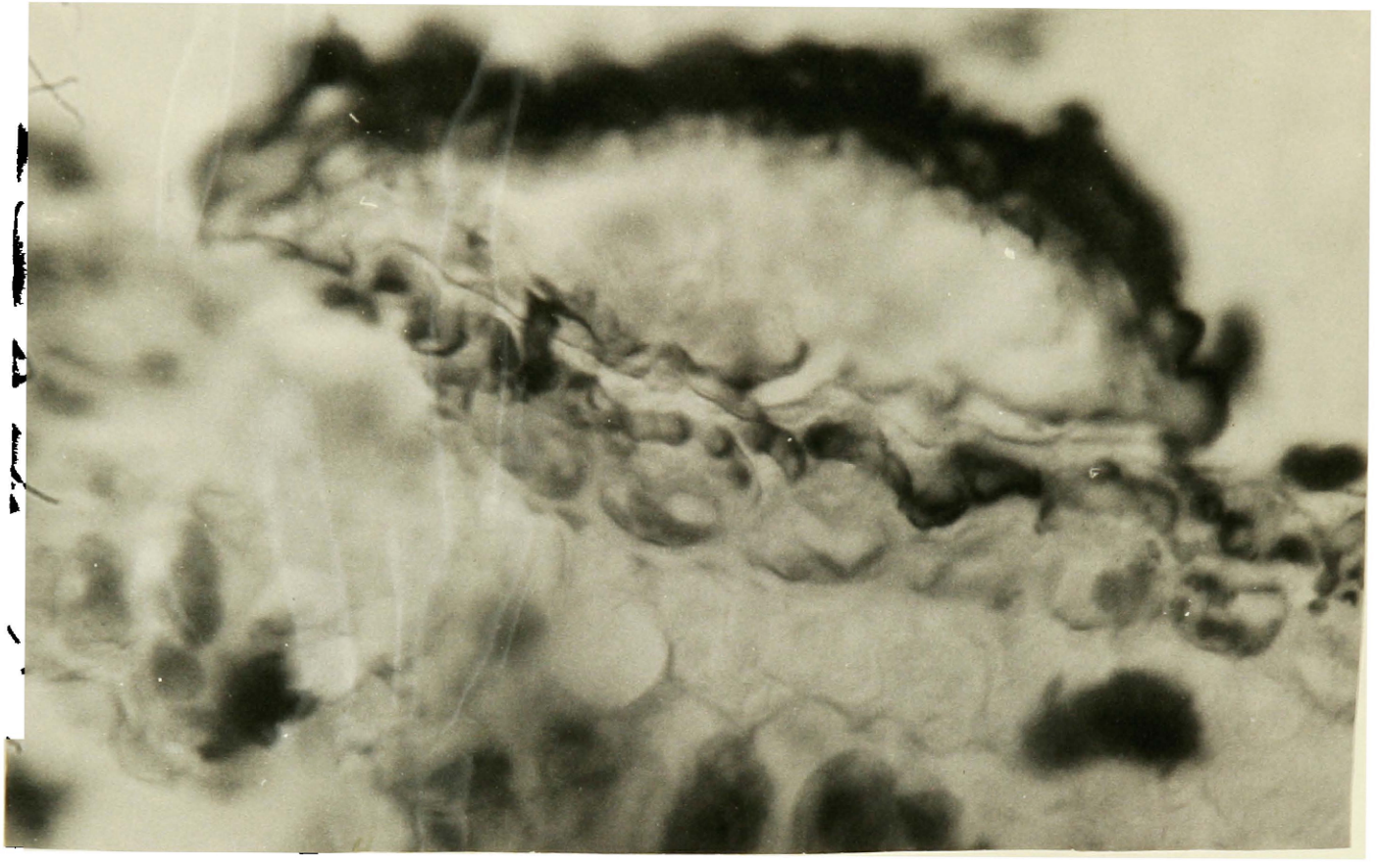

Fig. 15

Cross-section of ascocarp of Seynesia Juniperi on Juniperus californica.

(x 500)

Fig. 16

Ascocarps of Seynesia Juniperi on leaves of Juniperus californica. (From the herbarium of the Univ. of Calif. #790. Microthyrium Juniperi (desm.) Sacc.)

(x 75)

Fig. 17

Top view of ascocarps of Stigmatea sequoiae. Note the fimbriate borders.

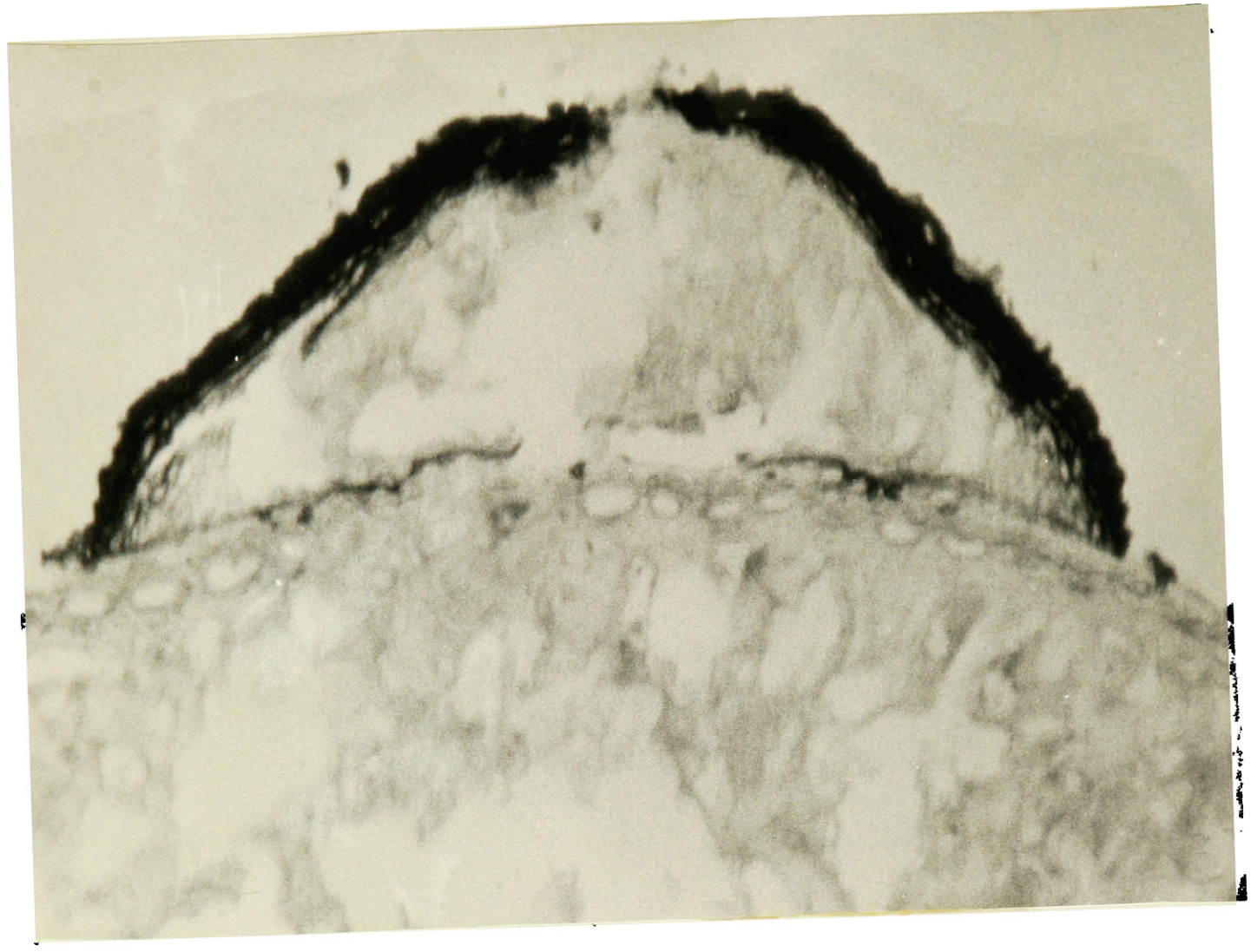

Fig. 18

Cross-section of ascocarp of Stigmatea sequoiae on Libocedrus decurrens.

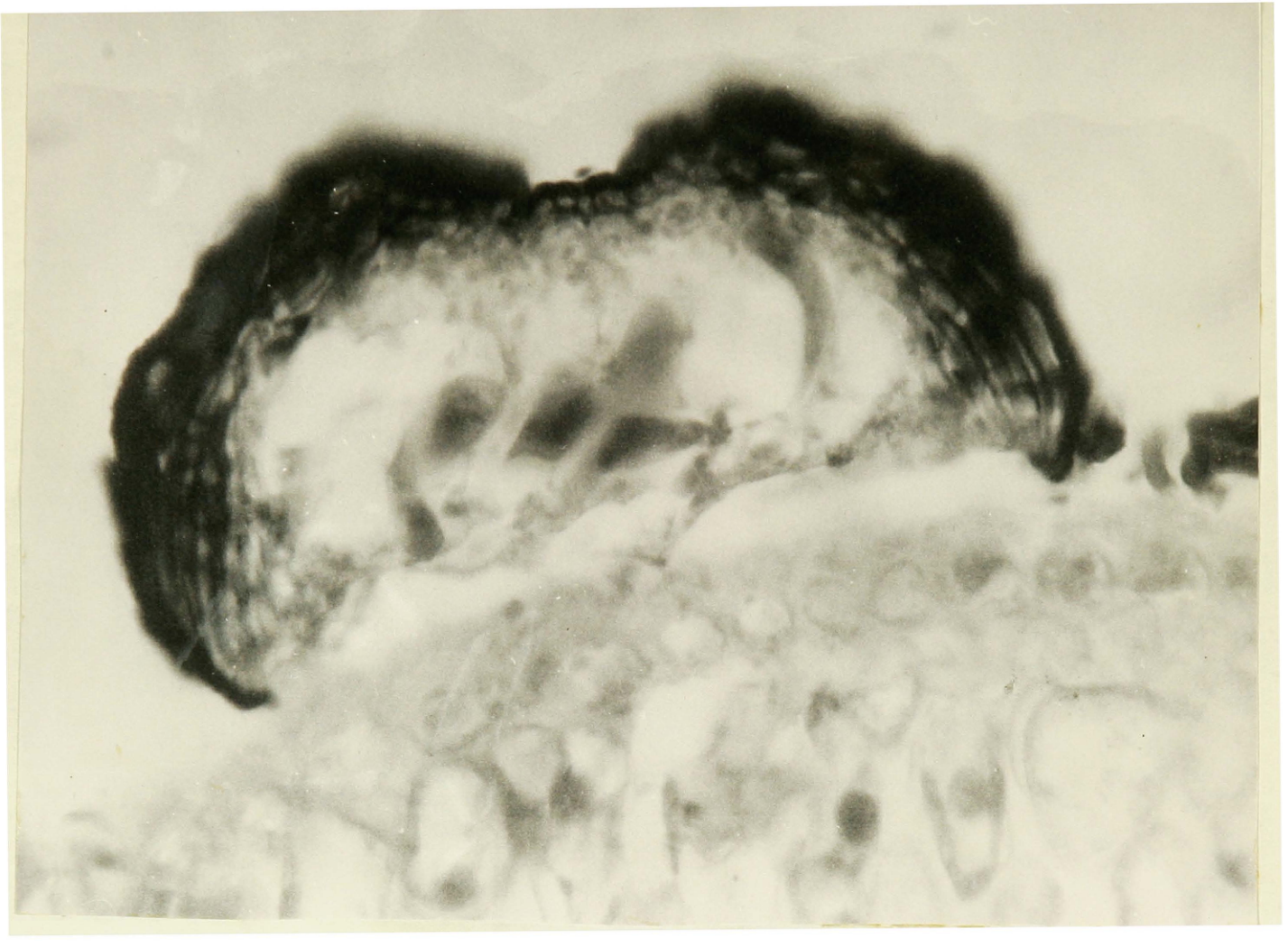

Fig. 19

Cross-section of ascocarp of Seynesia sp. nov.
Note the formation of the pseudo-ostiole.
(x 750)

Fig. 20

Lightly-infected branchlet of Juniperus scopulorum.
(x 45)

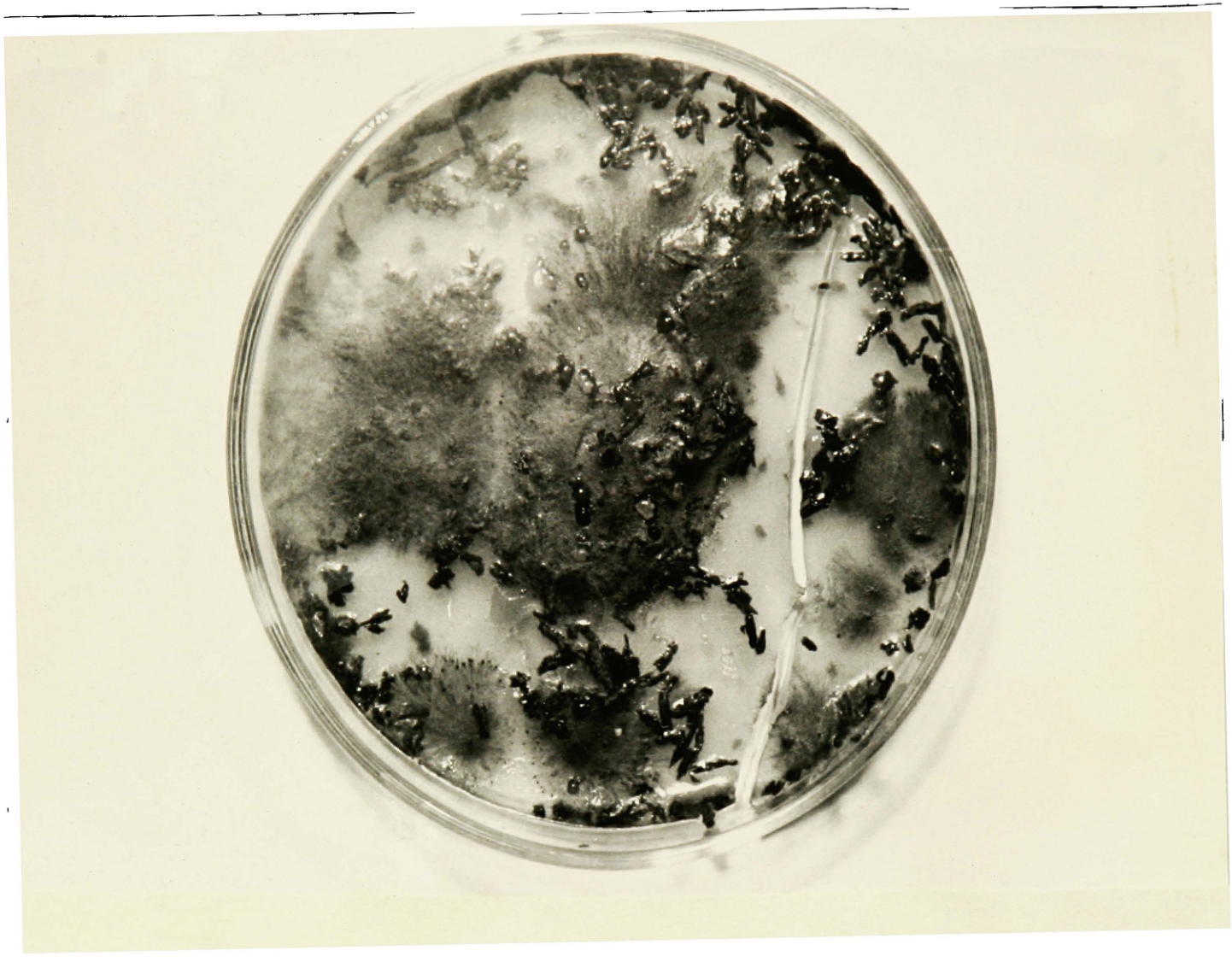

Fig. 21

Pycnidial growth in culture. Medium is 15% agar to which has been added bits of the branchlets of Juniperus scopulorum.